ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2018 September 14; 9(5): 71-118

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Volume 9 Number 5 September 14, 2018

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World Journal of Clinical Oncology (*WJCO*) is now abstracted and indexed in PubMed, PubMed Central, Scopus, and Emerging Sources Citation Index (Web of Science), China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

EDITORIAL BOARD MEMBERS
All editorial board members resources online at http://www.wjgnet.com/2218-4333/editorialboard.htm

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PUBLICATION DATE
September 14, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

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http://www.f6publishing.com

World Journal of Clinical Oncology

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World J Clin Oncol 2018 September 14; 9(5): 71-73

DOI: 10.5306/wjco.v9.i5.71

ISSN 2218-4333 (online)

EDITORIAL

Cancer prevention in patients with human immunodeficiency virus infection

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Author contributions: Valanikas E drafted the editorial; Dinas K and Tziomalos K critically revised the draft.

Conflict-of-interest statement: All authors declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

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Received: May 2, 2018
Peer-review started: May 3, 2018
First decision: June 6, 2018
Revised: June 16, 2018
Accepted: June 27, 2018
Article in press: June 28, 2018

Published online: September 14, 2018

Abstract

Cancer is a leading cause of death in patients with human immunodeficiency virus (HIV) infection. With the advent of antiretroviral treatment, the risk of AIDS-defining cancers declined but the ageing of this population resulted in the emergence of other common cancers, particularly lung and hepatocellular cancer. Accordingly, screening programs similar to the general population should be implemented in patients with HIV infection. Vaccination against common oncogenic viruses is also essential. However, rates of cancer screening and vaccination against HPV and HBV are considerably low in this population, highlighting a pressing need to educate patients and healthcare professionals about the importance of cancer preventive measures in these vulnerable patients.

Key words: Antiretroviral treatment; Prevention; Human immunodeficiency virus infection; Cancer; Vaccination

Core tip: Cancer is a leading cause of death in patients with human immunodeficiency virus (HIV) infection. With the advent of antiretroviral treatment, the risk of AIDS-defining cancers declined but the ageing of this population resulted in the emergence of other common cancers, particularly lung and hepatocellular cancer. Accordingly, screening programs similar to the general population should be implemented in patients with HIV infection. Vaccination against common oncogenic viruses is also essential.

Valanikas E, Dinas K, Tziomalos K. Cancer prevention in patients with human immunodeficiency virus infection. *World J*

Clin Oncol 2018; 9(5): 71-73 Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/71.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.71

Cancer is a leading cause of death in patients with human immunodeficiency virus (HIV) infection[1,2]. In recent decades, overall mortality declined in this population in industrialized countries but the percentage of deaths due to non-AIDS related cancer increased and currently represent almost one fourth of all deaths[1,2]. Moreover, HIV infection is associated with increased incidence of several cancers, including Kaposi's sarcoma, certain types of aggressive B-cell lymphomas and invasive cervical cancer, which are classified as AIDS-defining cancers[3-5]. However, several non-AIDS related cancers, including lung and hepatocellular cancer, are also observed more frequently in patients with HIV infection[3,6]. Therefore, the prevention of cancer is of paramount importance in this population.

The introduction of antiretroviral treatment (ART) resulted in substantial reductions in the incidence of AIDS-defining cancers[3-5]. Moreover, immunosuppresion also increases the risk of non-AIDS-defining cancers in this population[7,8]. Therefore, timely implementation of ART is essential for cancer prevention in patients with HIV infection. However, the cost/health benefit ratio of early implementation of ART and persistent suppression of HIV replication should also be considered, particularly in resource-poor settings.

It has been reported that almost 40% of cancers that affect patients with HIV infection are due to oncogenic virus, specifically hepatitis B and C virus infection-related hepatocellular carcinoma (HCC) and human papillomavirus (HPV) infection-related cervical, vulvar, penile, anal, oral and pharyngeal cancer[8,9]. Accordingly, vaccination against hepatitis B is recommended in all seronegative patients with HIV infection and repeat doses should be administered until anti-HBs titers $\geqslant$ 10-100 IU/mL are achieved[10]. Double doses might be indicated in patients with low CD4 count and high HIV viral load[10]. Vaccination against HPV is also recommended in patients with HIV infection < 26-year-old or < 40-year-old in men who have sex with men (MSM)[10]. Three doses of the 9-valent HPV vaccine should be used where available[10].

Lung cancer is more frequent in patients with HIV infection and is a leading cause of death in this population[3,6]. The higher prevalence of smoking in patients with HIV infection might partly contribute to this association[11,12]. Therefore, current guidelines state that these patients should be made aware of the detrimental effects of smoking on health and smokers should be informed about the benefits of smoking cessation[10]. For those willing to quit smoking, pharmacotherapy (including nicotine replacement therapy, varenicline and bupropion), cognitive behavioral counseling and/or motivational strategies can be employed to help quitting[10]. On the other hand, computed tomography screening appears to have low yield in patients with HIV infection, probably due to the young age of most of these patients[13].

Cancer screening recommendations in patients with HIV infection are similar to the general population, since there are no studies that specifically evaluated the benefits and harms of these strategies in this population[10]. Mammography is recommended every 1-3 years in women 50-70-year-old and measurement of prostate specific antigen is recommended every 2-4 years in men > 50 years with life expectancy > 10 years[10]. Annual faecal occult blood test, sigmoidoscopy every 5 years or colonoscopy every 10 years are recommended in subjects > 50 years with life expectancy > 10 years[10]. In patients with cirrhosis and in those with HBV co-infection and either a history of elevated transaminases or risk factors for HCC (family history of HCC, Asians, Africans), abdominal ultrasound and measurement of alpha-fetoprotein levels are recommended every 6 mo to enable the early diagnosis of HCC[10]. Regarding AIDS-defining cancers, liquid-based cervical cytology test every 1-3 years is recommended in women > 21 years or within 1 year after sexual debut[10]. Digital rectal examination with or without anal cytology is also recommended in MSM and patients with HPV-associated dysplasia[10]. Finally, careful inspection of the skin should be performed regularly to detect cancers such as Kaposi's sarcoma, basal cell carcinoma and malignant melanoma[10].

In conclusion, cancer is a frequent cause of death in patients with HIV infection. With the advent of ART, the risk of AIDS-defining cancers declined but the ageing of this population resulted in the emergence of other common cancers, particularly lung cancer and HCC. Accordingly, screening programs similar to the general population should be implemented in patients with HIV infection. Vaccination against common oncogenic viruses is also essential. In addition, primary prevention of cancer by implementing educational programs stressing the importance of healthy lifestyle are equally important in patients with HIV infection. However, rates of cancer screening and vaccination against HPV and HBV are considerably low in this population[14,15], highlighting a pressing need to educate patients and healthcare professionals about the importance of cancer preventive measures in these vulnerable patients.

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P- Reviewer: Fiorentini G, Scaggiante B, Syed V, Yang T, Zhong ZH
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Tan WW

World Journal of Clinical Oncology

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World J Clin Oncol 2018 September 14; 9(5): 74-82

DOI: 10.5306/wjco.v9.i5.74

ISSN 2218-4333 (online)

REVIEW

Oxytocin and cancer: An emerging link

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Author contributions: Lerman B and Ogunwobi OO conceived of the article; Lerman B, Harricharran T and Ogunwobi OO wrote the article; Ogunwobi OO supervised all of the work.

Conflict-of-interest statement: The authors have no conflicts of interest.

Manuscript source: Invited manuscript

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Received: June 11, 2018
Peer-review started: June 12, 2018
First decision: July 9, 2018
Revised: July 11, 2018
Accepted: August 6, 2018
Article in press: August 7, 2018
Published online: September 14, 2018

Abstract

The neuropeptide hormone oxytocin, which is released from the posterior pituitary gland, is involved in a number of physiological processes. Understanding of its effects is gradually increasing due to new research in this area. While mostly recognized as a reproductive system hormone, oxytocin also regulates other organ systems such as the brain and cardiovascular system. Recently, research has focused on unraveling its involvement in cancer, and emerging evidence suggests a potential role for oxytocin as a cancer biomarker. This review summarizes observations linking oxytocin and cancer, with a special emphasis on prostate cancer, where it may promote cell proliferation. Research suggests that oxytocin effects may depend on cell type, concentration of the hormone, its interactions with other hormones in the microenvironment, and the precise localization of its receptor on the cell membrane. Future research is needed to further elucidate the involvement of oxytocin in cancer, and whether it could be a clinical cancer biomarker or therapeutic target.

Key words: Oxytocin; Cancer; Prostate; Pancreas; Exercise

Core tip: Oxytocin's role outside of the reproductive system and social bonding has yet to be fully elucidated. Apparently, its role in cancer may vary depending on location and cell type. This review summarizes the current state of our understanding of the potential role of oxytocin in cancer.

Lerman B, Harricharran T, Ogunwobi OO. Oxytocin and cancer: An emerging link. *World J Clin Oncol* 2018; 9(5): 74-82

Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/74.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.74

INTRODUCTION

Oxytocin is a central nervous system (CNS) neuropeptide hormone, which is composed of nine amino acids. The synthesis of oxytocin begins in the hypothalamus, where the paraventricular nucleus and supra-optic neurons express high levels of oxytocin, which is released from the posterior pituitary gland[1]. Oxytocin is biologically similar to vasopressin (also known as antidiuretic hormone), and they are often studied in parallel, as both hormones also share some functions. Originally thought of as a hormone with a role limited to the uterus and milk ejection - oxytocin means "quick birth" in Greek[2] - further research has expanded understanding of its function across sexes and organ systems. Furthermore, it has become clear that in addition to physical function, oxytocin also wields important impact on social behaviors, which include stress and trust, anxiety, social interaction and bonding, and parental care[3], and thereby on neuropsychiatric disorders linked to these social behaviors. Interestingly, emerging evidence has linked oxytocin to somewhat conflicting roles in carcinogenesis, as oxytocin is implicated in either fostering development or, conversely, inhibition of cancer-related cellular functional phenomena.

PHYSIOLOGICAL FUNCTIONS OF OXYTOCIN

Oxytocin in the reproductive system

Oxytocin exerts its effects primarily through a single receptor, which has been well characterized. The oxytocin receptor is a class- Ⅰ G-protein-coupled receptor with seven transmembrane domains, and can be bound by several ligands, including oxytocin, oxytocin agonists and antagonists, as well as vasopressin[4]. These receptors are found in the endometrium, myometrium, trophoblast, osteoblasts, reproductive organs, and throughout the CNS (Table 1). Notably, oxytocin receptor has also been implicated in various cancers related to tissues in which it is expressed, including endometrial cancer, glioblastomas, neuroblastomas[5], and others. Several oxytocin receptor antagonists have been identified, with the most common being Atosiban[6,7]. While studies of oxytocin antagonist in cancer has been limited, atosiban has been implicated in inhibiting cell growth of DU145 prostate cancer[8] and various breast cancer cell lines[9]. However, little evidence indicates whether this strategy is efficacious *in vivo*.

The effectors of the oxytocin receptor may vary. While the primary signaling mechanisms of oxytocin have not been fully elucidated, recent studies show that the main mitogenic signaling mechanism of the oxytocin receptor involves the Gq alpha subunit protein (Gαq)/phospholipase C (PLC)/inositol 1, 4, 5 triphosphate (InsP3) pathway. Through this activation, the G protein couples to PLC-B, resulting in the release of calcium from intracellular stores[10], triggering smooth muscle contractions[11], for example in the uterus or in the myoepithelial cells of the mammary gland. The main oxytocin pathways described in this paper are depicted in Figure 1.

Indeed, the first functional role attributed to oxytocin centered on the female reproductive system, specifically in uterine contraction and in lactation. Uterine sensitivity to oxytocin increases around the onset of labor, and upon labor oxytocin stimulation becomes more efficient. Therefore, exogenous administration of oxytocin is also clinically used to induce labor. Following parturition, the density of oxytocin receptors are declines. In lactation, the oxytocin pathway is activated when the infant begins sucking on the nipple[12]. A sensory impulse is sent from the nipple to the spinal cord, and from there transmitted to the oxytocinergic neurons in the hypothalamus. These neurons generate action potentials that lead to a substantial release of oxytocin into the blood stream, which subsequently elicits milk ejection via a contraction of myoepithelial cells[4]. Interestingly, this cascade can be triggered even before suckling occurs, by an event such as a baby crying[13], suggesting that it may involve reflex neural pathways. Increased plasma oxytocin has also been linked to an increased risk of pregnancy-induced hypertension[14], and exogenous oxytocin administration has been associated with angiotensin II-induced hypertension[15]. Interestingly, though, there is also a potential link between increased oxytocin and reduced risk of hypertension (which might be acquired *in utero* in association with intrauterine growth retardation)[16], so that oxytocin has been attributed with eliciting a reduction in blood pressure[17,18].

The reproductive function of oxytocin is not limited to females, as it stimulates contractility of the seminiferous tubules, epididymis, and the prostate gland[19]. Due to its production locale in the testes, oxytocin has been studied as a paracrine regulator of the prostate gland, specifically of growth and muscle contractility[20]. In males, oxytocin has been thought to induce erection and play a role in ejaculation[19,21]. Specifically, in the prostate, oxytocin has been suggested to induce prostatic smooth muscle cell contraction[22]. Its postulated involvement in ejaculation includes stimulation of the reproductive tract to promote sperm release[19]. Oxytocin's role in aggravating and potentially facilitating the development of benign prostatic hyperplasia, and the oxytocin-induced proliferative effect, are likely mediated through the extracellular signal regulated kinase (ERK) pathway[23].

Oxytocin in the CNS

Given its ubiquitous distribution in the CNS, the role of oxytocin in cognition and social behavior has also been studied extensively, especially over the past decade.

Table 1 Human tissue with known expression of the oxytocin receptor

Tissue	Expression type
Myometrium	mRNA and protein[7,11]
Lung	mRNA[109]
Breast	mRNA and protein[16]
Prostate	mRNA[22,79]
Uterus	mRNA and Protein[11]
Heart	mRNA[17]
Vascular endothelium	mRNA[110]
Brain	mRNA[31,42,43]
Thymus	mRNA[111]
Pancreas	mRNA[112]
Blood	mRNA and protein[113]
Bone	mRNA[114]

It has been shown that oxytocin can enhance positive social interactions, and importantly can enhance trust[24]. Oxytocin activity was found to be decreased in women who suffered abuse in their youth[25]. Conversely, and perhaps related to this, a study reported increased oxytocin levels in individuals enjoying heightened levels of partner support[26]. Oxytocin also has been reported to improve social cognition[27,28]. However, it must be noted that studies on the social effects of oxytocin are somewhat inconsistent[29], suggesting that at least socially, additional factors are likely to be at play.

One of the aims of oxytocin research stems from an attempt to establish it as a tool to predict, diagnose, and potentially treat neuropsychiatric disorders[30], and has mostly focused on anxiety and depression. Oxytocin intake has been shown to reduce anxiety symptoms[31,32]. In concordance with its pivotal role during birth, the majority of the research regarding depression in humans has revolved around pregnancies and the mother's ability to recover from postnatal depression (PND). Studies in this field have discovered lowered oxytocin in mothers with PND, and that increased oxytocin levels bestow positive effects on mothers with PND and on their interactions with infants[33,34]. On the other hand, abnormal post-prandial oxytocin secretion has also been demonstrated in women with anorexia nervosa, possibly as an adaptive response to food-related symptoms of anxiety and depression[35]. One of the emerging "hot topics" in neuropsychiatric research links oxytocin with autism[36-38], with recent studies beginning to identify oxytocin as a potential medical therapy to alleviate social anxiety caused by autism[39].

Oxytocin in the cardiovascular system

The potential regulatory role of oxytocin in other organ systems has also raised considerable interest. Oxytocin contributes to several forms of cardiovascular regulation, as it has been shown that preconditioning rats with oxytocin reduces cardiac arrhythmias[40], and that oxytocin can lower blood pressure[41], increase anti-inflammatory and antioxidant activity, and exert beneficial metabolic effects. Therefore, its cardiovascular activity seems to aim largely at restoring homeostasis.

Notably, oxytocin seems to also exert cardiovascular regulation during elevated levels of physical activity. Oxytocin levels have been shown to rise in response to exercise[42], which activates oxytocinergic projections[43] and oxytocinergic modulatory loops that adjust cardiac output, assisting in keeping cardiovascular control over the blood supply[44]. The rise in oxytocin has been traced to the lumbar spinal cord[45]. Oxytocin also reduces the rise of exercise-induced adrenocorticotropic hormone (ACTH) and cortisol[46,47], furthering support on its effects on cortisol levels. Furthermore, exogenously administered oxytocin along with exercise have been shown to protect ovariectomized rats from myocardial infarction[48]. On the other hand, its contribution to cardiovascular regulation may depend on the type or intensity of exercise, because plasma oxytocin in cyclists remains unchanged during intense exercise[49].

OXYTOCIN IN CANCER

Less is understood about the connection between oxytocin and cancer, partly due to lack of adequate research in this area, and partly due to some inconsistency in the current data (Figure 2). The first link of oxytocin to cancer was reported in 1984, when oxytocin was described to be structurally and genomically related to vasopressin, an endogenous hormone that is also secreted by the pituitary, and that in addition to its physiological functions has been found to constitute a biomarker of small-cell lung cancer[50]. Furthermore, oxytocin and vasopressin are co-expressed in these cells, where they have been proposed to induce mitogenic effects[51]. Oxytocin's link to vasopressin and its potential role as a biomarker was subsequently proposed in 1990[52]. Shortly thereafter, it was suggested that oxytocin may modulate growth in breast cancer[53], which was subsequently demonstrated[54]. These observations have instigated additional research into oxytocin's potential involvement in various forms of cancer.

Oxytocin in breast cancer

Interestingly, subsequent studies have shown that oxytocin in fact inhibits proliferation of breast cancer cell lines, such as MDA-MB231, MCF7, and T47D[55,56], as well as the canine mammary cell line CMT-U27[57], mouse mammary carcinoma cell line TS/A, and rat mammary carcinoma cell line D-R3230AC[9]. This effect was shown to be mediated via the cyclic adenosine monophosphate protein kinase A in human cell lines[58]. Importantly, anti-proliferative and tumor inhibitory properties were also observed *in vivo* in both rat and mouse experimental models, and attributed to both oxytocin and its analogue F314[9]. Recently, it was suggested that exercise training, by inducing oxytocin secretion, may reduce the expression of specific signaling proteins involved in breast cancer[59].

Lactation has long been linked to a reduced risk

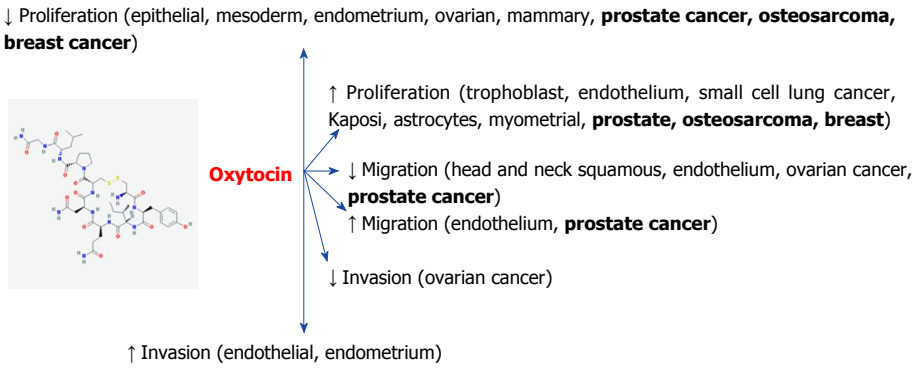

Figure 1 The potential role of oxytocin in various cancers and cell types. Bold font indicates conflicting observations.

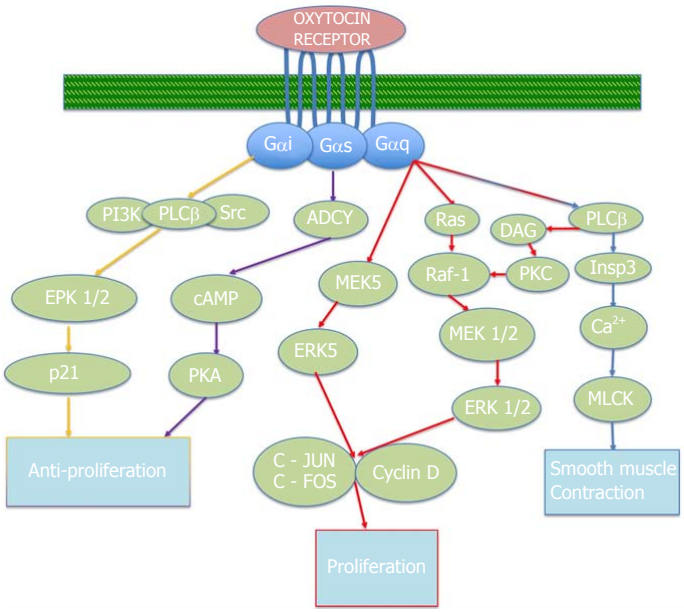

Figure 2 Mechanisms of action of oxytocin. Yellow and purple are anti-proliferation (*via* different subunits), red is proliferation, and blue is smooth muscle contraction. Gαi: Guanine nucleotide binding protein subunit alpha I; Gαs: Guanine nucleotide binding protein subunit alpha s; Gαq: Guanine nucleotide binding protein subunit alpha q; PI3K: Phosphoinositide 3-kinase; PLCβ: Phospholipase beta; Src: Tyrosine protein kinase; ERK: Extracellular signal-regulated kinase; p21: Cyclin-dependent kinase inhibitor 1; ADCY: Adenylate cyclase; cAMP: Cyclic AMP; PKA: Protein kinase A; DAG: Diacylglycerol; PKC: Protein kinase C; Insp3: Inositol triphosphate 3; MLCK: Myosin light chain kinase.

of cancer, with research dating back to as early as the 1950's[60-63]. Worldwide, it has been shown that breastfeeding reduces the risk of both breast and uterine cancer, with prolonged durations of breastfeeding (usually involving multiple children breastfed) correlating with a progressive fall in the risks of both breast and uterine cancer[64-68]. The relationship with uterine cancer might be related to the action of oxytocin as a paracrine and endocrine hormone in lactation. Nevertheless, while the relationship between oxytocin, lactation, and breastfeeding with reduced risk of breast and uterine cancer are all well documented individually, more research needs to be conducted to determine if the relationship between oxytocin, lactation, and breastfeeding with reduced breast and uterine cancer is causal. Elucidating such a connection may establish new therapeutic targets in cancer.

Oxytocin in ovarian cancer

In addition to breast and uterine cancer, the potential participation of oxytocin in the pathogenesis of other cancers in the reproductive system has been investigated. Oxytocin was found to inhibit the progression of ovarian carcinoma (Figure 2) both *in vitro* and *in vivo*. Using cell viability, invasion, and migration assays, it was demonstrated that oxytocin inhibited proliferation, migration and invasion of ovarian cancer cells *in vitro*, and its administration also attenuated the dissemination of ovarian cancer using mean tumor burden as a measure[69]. The same investigators had demonstrated in a previous study expression of the oxytocin receptor in various human ovarian carcinoma tissues and cell lines, and identified placental leucine aminopeptidase (P-LAP) as an oxytocin-degrading oxytocinase in certain adenocarcinoma tissues[70]. This team of investigators,

therefore, proposed that a system involving P-LAP and oxytocin plays a role in the regulation of human endometrial adenocarcinoma, in which P-LAP exerts a functionally positive impact on carcinoma cell growth by degrading suppressive peptides such as oxytocin. More recently, these effects have also been linked with a cross-talk network between oxytocin and the stress hormone cortisol, whereby oxytocin reversed the carcinogenic effects of cortisol via autophagy (cellular self-degradation)[71]. Interestingly, pertinent to the postulated connection between oxytocin and symptoms of autism[39], oxytocin and cancer have also demonstrated an inverse relationship in autistic children[72].

Oxytocin in the gastrointestinal tract

Oxytocin receptors are expressed throughout the gastrointestinal (GI) tract[73], but little is known about their function in the GI tract, especially in relation to cancer. Some studies have suggested a link between oxytocin and its receptor in GI-related cancers, such as esophageal, gastric, and pancreatic cancers. For example, some studies showed an inverse relationship between the duration of breastfeeding and risk of esophageal cancer[74,75], gastric cancer[76], and pancreatic[77] cancer. In fact, Yu *et al*[78] showed a 54% decreased risk of developing esophageal cancer in women who breastfed for over 12 mo.

Unpublished data from our laboratory shows that the messenger ribonucleic acid (mRNA) expression of oxytocin is twofold higher in PANC-1 (a human pancreatic cancer cell line highly unresponsive to the chemotherapeutic agents, gemcitabine and 5-FU) compared to L3.6pl (a highly responsive human pancreatic cancer cell line). We also found that oxytocin receptor protein expression is also higher in PANC-1 than in L3.6pl. Further, inhibition of the oxytocin receptor decreased cell proliferation of PANC-1 and L3.6pl cells. Our analysis of data from the cBioPortal database revealed that up to 5% of pancreatic cancer patients included in The Cancer Genome Atlas showed genetic alterations (primarily upregulation of mRNA expression) in oxytocin and its receptor. Patients with these alterations had poorer survival outcomes as compared to those without these alterations. These interesting data warrant further investigation on the molecular mechanisms implicating oxytocin and its receptor in pancreatic cancer and other GI cancers.

Oxytocin in prostate cancer

As a role for oxytocin in the regulation of prostate function is established, its potential involvement in the development of prostate cancer has been proposed. Data from over two decades ago implicated oxytocin in the pathophysiology of benign prostatic hyperplasia, where the peptide might contribute to both the physical enlargement and dynamic tone of the gland[19]. More recently, immunohistochemical staining has detected oxytocin expression in stromal and epithelial cell lines and in tissue from patients with benign prostatic hyperplasia, which was significantly reduced in tissues of invasive prostate cancer in comparison to both benign prostatic hyperplasia tissues and normal human prostate epithelial cells[79]. This inverse relationship might implicate a fall in oxytocin levels in progression of prostate cancer. Within the prostate, oxytocin has been shown to affect gland growth both directly and *via* its interaction with androgen metabolism, and oxytocin concentrations are positively correlated with androgens[20,80]. Indeed, while in the absence of androgens oxytocin had no effect on prostate cancer cell lines (LNCaP and PC-3), in the presence of testosterone low oxytocin doses stimulated proliferation of PC-3 cells[81], supporting the notion that changes in levels of oxytocin in the prostate in aging and cancer may promote prostate epithelial cell proliferation. It is possible that increased levels of oxytocin might be involved in the mechanisms by which high ejaculation frequency is related to decreased risk of prostate cancer[82]. This hypothesis needs to be further investigated.

Conversely, a different study recently revealed that oxytocin increased the expression of APPL1, a protein with the ability to interact with tumor suppressor proteins. *In vitro* studies showed that oxytocin increased prostate cancer cell proliferation, and expression of APPL1. Analysis of serum and tissue samples identified increased oxytocin levels in the serum of prostate cancer patients, and high expression of oxytocin and its receptor in prostate tissues collected from prostate cancer patients in comparison to those collected from patients without prostate cancer. The oxytocin receptor has also been implicated in the migration of prostate cancer cells, and possibly modulation of prostate cancer metastasis[83]. Taken together, these observations of oxytocin in prostate cancer cells both *in vivo* and *in vitro,* suggest that oxytocin could serve as a prostate cancer biomarker[84].

Several explanations have been offered for the apparent differences in the data from different studies regarding the role of oxytocin in prostate cancer. One explanation is the notable difference in the numbers of participants involved in each study. Secondly, some of the studies included prostate cancer patients that had undergone neo-adjuvant therapy, which can affect oxytocin levels[85]. Thirdly, oxytocin is likely to activate a wide range of signaling mechanisms to elicit variable cellular responses, possibly depending on the density or precise localization of the oxytocin receptor on the plasma membrane[86]. This may also account for the dichotomy in the observations reported regarding the role of oxytocin in cancer. Clearly, additional studies are needed to elucidate the involvement of oxytocin and oxytocin receptor in progression or regression of human prostate cancer.

Perspectives and future directions

There is clearly some evidence implicating oxytocin in carcinogenesis, although its precise effect and underlying mechanisms are still unclear. It is possible that in some

individuals, cell types, or types of cancers, oxytocin may not act as a sole regulator of carcinogenesis, but may mediate or modulate other coexisting factors in the microenvironment.

For example, research generally supports the hypothesis that exercise can inhibit the progression of cancer[87-89]. The positive impact of exercise in blunting development of cancer and facilitating recovery may potentially be partly mediated through oxytocin. For example, oxytocin has been proposed to mediate an exercise-induced reduction in the expression of specific signaling molecules involved in breast cancer[59]. It has also recently been shown *in vivo* that the combination of exogenous oxytocin with exercise improves cardiac function, which might be associated with improved cancer survival. Cardiac dysfunction and cancer have such a strong link that it has even spawned its own subspecialty in cardio-oncology[90-93], although much of the research has centered around long-term survival and cardiac complications in cancer patients. While this potential mediating effect of oxytocin has been hypothesized[94], it appears that little primary research has been conducted to address this postulation. The ability to knockout or silence oxytocin is available, and its social effects are already documented[95,96]. Thus, a thorough study is certainly possible. Similarly, oxytocin may mediate the inhibitory effects of lactation on development of breast cancer, and of ejaculation on development of prostate cancer, and additional studies could prove to be invaluable in revealing its involvement.

It must be noted that most systems and bodily processes in living organisms are tightly inter-connected. Untangling this complexity in the presence of confounding elements is often difficult, especially in relation to psychosocial factors. Therefore, interpretation of oxytocin's expression and levels alone might be over-simplistic and under-informative. For example, instead of being increased as a direct response to exercise, oxytocin may be induced to assist its "companion" hormone, vasopressin, a well-known hormonal regulator of body fluid homeostasis[97]. Cortisol, the "stress hormone" and the rhythm surrounding its release has been linked to the progression and survival from various cancers[98-101], and has also been linked to oxytocin[71,72]. The ability to completely elucidate various pathways and mechanisms of actions would go a long way to showing if the established connection between the different hormones extends to cancer. Furthermore, while outside the scope of this review, there are several other diseases and pathologic states possibly linked to oxytocin. Pain, depression, and anxiety have all been linked to oxytocin[102,103]. Oxytocin's role in depression management was mentioned previously in this article, but oxytocin also seems to be a promising target in pain management[104-107], and in immunotherapy, especially through its interactions in the gut[108].

CONCLUSION

Most knowledge of oxytocin centers on its role as a reproductive hormone. Since its discovery, its other roles have progressively become clearer, including involvement in social behavior, cardiovascular regulation, and carcinogenesis. While it is currently difficult to pinpoint and precisely define oxytocin's oncogenic roles, it is hoped that this review will encourage greater intensity in researching the details of the role of oxytocin in cancer. Future research has a number of plausible and exciting directions to follow and will hopefully clarify some of the ambiguities concerning the role of oxytocin in cancer.

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P- Reviewer: Hosseini M, Vinh-Hung V, Wion D **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu YXJ

World Journal of Clinical Oncology

Submit a Manuscript: http://www.f6publishing.com

World J Clin Oncol 2018 September 14; 9(5): 83-89

DOI: 10.5306/wjco.v9.i5.83

ISSN 2218-4333 (online)

REVIEW

Role of polymorphisms in genes that encode cytokines and *Helicobacter pylori* virulence factors in gastric carcinogenesis

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Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

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Received: April 30, 2018
Peer-review started: April 30, 2018
First decision: May 17, 2018
Revised: June 23, 2018
Accepted: June 27, 2018
Article in press: June 28, 2018
Published online: September 14, 2018

Abstract

The *Helicobacter pylori* (*H. pylori*) infection is a determinant factor in gastric cancer (GC) development. However, the infection outcomes are variable and depend on both host and bacterial characteristics. Some host cytokines such as interleukin (IL)-1β, IL-1Ra, IL-8, IL-10 and tumor necrosis factor-α play important roles in the host immune system response to the pathogen, in the development of gastric mucosal lesions and in cell malignant transformation. Therefore, these host factors are crucial in neoplastic processes. Certain polymorphisms in genes that encode these cytokines have been associated with an increased risk of GC. On the other hand, various virulence factors found in distinct *H. pylori* bacterial strains, including cytotoxin-associated antigen A, vacuolating cytotoxin, duodenal ulcer promoting gene A protein, outer inflammatory protein and blood group antigen binding adhesin, have been associated with the pathogenesis of different gastric diseases. The virulent factors mentioned above allow the successful infection by the bacterium and play crucial roles in gastric mucosa lesions, including malignant transformation. Moreover, the role of host polymorphisms and bacterial virulence factors in gastric carcinogenesis seems to vary among different countries and populations. The identification of host and bacterium factors that are associated with an increased risk of GC development may be useful in determining the prognosis of infection in patients, what could help in clinical decision-making and in providing of an optimized clinical approach.

Key words: *Helicobacter pylori*; Virulence factors; Cytokines; Gene polymorphisms; Gastric cancer

Core tip: Various polymorphisms in host genes that encode cytokines and *Helicobacter pylori* virulence factors have been associated with different tendencies of gastric diseases development. Several reviews have been written on the role of host and bacterial isolated factors in gastric carcinogenesis. However, only a small amount of reviews unites the important characteristics of both bacterium and host in carcinogenesis. General overviews about polymorphisms in genes that encode cytokines are also scarce. We aimed to join the main polymorphisms in genes that encode cytokines and bacterial virulent factors related to gastric carcinogenesis and to provide a broad overview about these themes.

de Brito BB, da Silva FAF, de Melo FF. Role of polymorphisms in genes that encode cytokines and *Helicobacter pylori* virulence factors in gastric carcinogenesis. *World J Clin Oncol* 2018; 9(5): 83-89 Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/83.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.83

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram negative bacterium, which inhabits the gastric epithelial tissue of most people in the world[1], and it is considered a determinant factor in the initiation of gastric carcinogenesis[2]. Gastric cancer (GC) is one of the four most prevalent neoplasms and the second biggest cause of deaths in consequence of cancer worldwide[3]. Despite the importance of *H. pylori* in gastric carcinogenesis, the development of GC only occurs in a minority of infected people, demonstrating that the infection outcomes are variable. It is believed that multifactorial precancerous processes associated with both host mucosal inflammatory response and pathogen characteristics are determinant in the severity of the disease[4].

The host immune system response plays a crucial role in the outcomes of *H. pylori* infection. Polymorphisms in genes that encode cytokines have been reported and associated with the severity of gastric mucosa inflammation and GC development. Some of these determinant variations are present in genes that encode cytokines such as interleukin (IL)-1β, IL-1Ra, IL-8, IL-10 and tumor necrosis factor (TNF)-α[5-13]. These polymorphisms are important aspects in understanding gastric carcinogenesis, since chronic inflammation induced by the bacterium is critical in the emergence and evolution of GC precursor lesions (Figure 1)[14].

On the other hand, the virulence factors of *H. pylori* are determinant in the interaction with host cells. Cytotoxin associated antigen A (CagA), vacuolating cytotoxin (VacA), duodenal ulcer promoting gene A protein (DupA), outer inflammatory protein (OipA) and blood group antigen binding adhesin (BabA) are some virulent factors that seem to be associated with different risks of GC development[15]. Furthermore, *H. pylori* with EPIYA-D or more than one EPIYA-C segment in its *CagA* gene have been associated with a higher risk of gastric carcinogenesis[16-20].

POLYMORPHISMS IN GENES THAT ENCODE CYTOKINES AND GASTRIC CARCINOGENESIS

Gastric carcinogenesis is a process in which chronic inflammatory status plays a crucial role. The increase of inflammatory cytokine levels, due to *H. pylori* infection, seems to be determinant in the initiation and progression of GC[12]. The intensity of the expression of cytokines can be modified by functional polymorphisms in the promoter regions of the genes, which has the potential to alter the affinity of transcription factors, interfering in the expression levels of the messenger ribonucleic acid (mRNA) of specific inflammatory mediators related to the susceptibility of GC initiation[21].

IL-1

IL-1 is a family of cytokines that possesses 11 described members, among which IL-1β and IL-1 receptor antagonist (IL-1Ra), combined with *H. pylori* infection, seem to be key factors in GC development[22-24]. Signaling through the IL-1 receptor is a necessary event for the beginning and sustenance of various responses of the immune system[25].

The promoter regions of *IL1B* and *IL1RN* genes, which encode IL-1β and IL-1Ra respectively, have SNPs that modify the expression of the genes and affect the inflammatory response[26]. These SNPs increase the IL-1β/IL-1Ra ratio, which unleashes processes that result in gastric hypochlorhydria, favoring GC development[15,27].

IL-1β is an important cytokine for host-response to pathogens; however, this mediator can exacerbate damage during chronic diseases[28]. High levels of IL-1β in *H. pylori* infections lead to gastrin overexpression, increased gastric inflammation, hypochlorhydria, and gastric atrophy[29]. Moreover, IL-1β might promote neoplastic growth[30]. The *IL1B* gene can be composed by three different SNPs: C-T base transition at IL-1B-511 (rs16944), T-C base transition at IL-1B-31 (rs1143627) and IL-1B-3954 (rs1143634), and all of them are strongly associated with increased production of proinflammatory cytokines, hypochlorhydria and increased GC risk, mainly intestinal type, among Caucasians, but not among Asians or Hispanics[31-34].

IL-1Ra inhibits IL-1α and IL-1β by means of binding to IL-1 receptors. IL1RN possesses a changeable number of tandem repeats in intron 2, forming long alleles (IL1RN1) with 3-6 repeats or a short allele (IL1RN2) with 2 repeats[35]. The IL1RN2 allele is associated with severe gastric lesions and higher risk for GC, besides raised IL-1β expression in Caucasians[33-36].

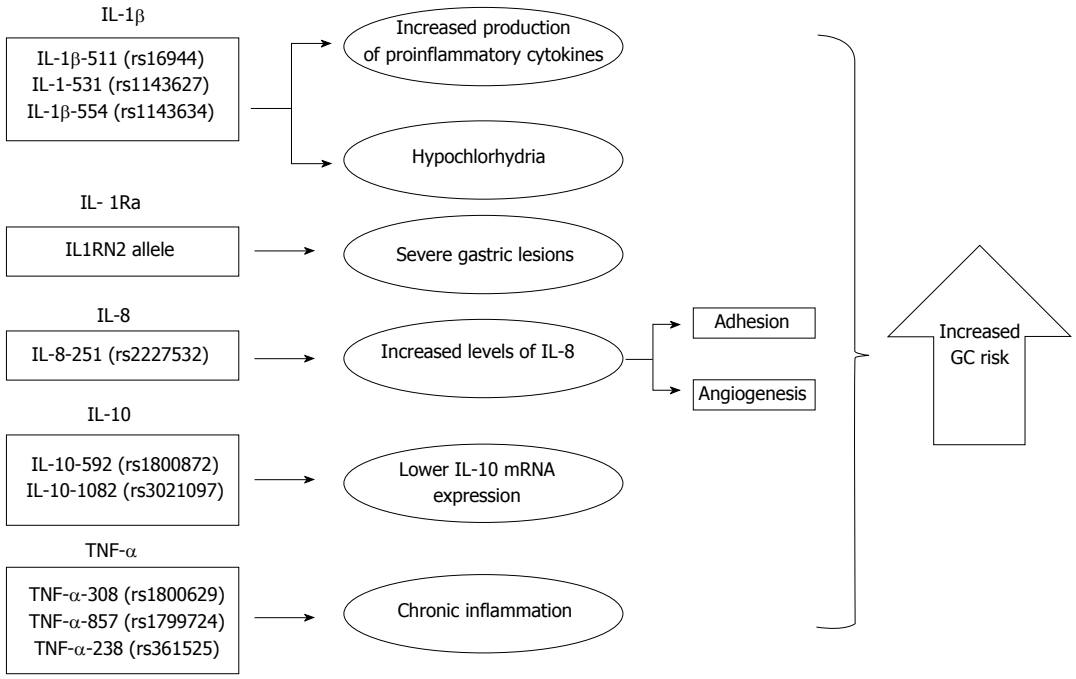

Figure 1 Potential functions of the host genetic polymorphisms in gastric carcinogenesis. IL: Interleukin; GC: Gastric cancer; TNF: Tumor necrosis factor.

IL-8

IL-8 is a potent cytokine that induces the directed migration of cells to inflammatory sites, acting as a chemoattractant[37]. IL-8 secretion can be increased by different stimuli, such as live bacteria (including *H. pylori*) and lipopolysaccharides (LPS), besides others inflammatory cytokines, including IL-1 and TNF[38]. The association of IL-8 with angiogenesis, adhesion and tumorigenesis have been related[39,40].

The gene *CXCL8*, which encodes IL-8, is located on 4q12-21 chromosome and possesses four exons and three introns[41]. An A/T SNP in the -251 position of this gene (*rs2227532*) has been associated with the development of various inflammatory diseases and cancer, including GC in Asians, but not in Europeans[42,43]. Furthermore, the IL-8-251 A allele was related to increased levels of IL-8[41].

IL-10

In opposition to the cytokines mentioned above, IL-10 is an anti-inflammatory cytokine, and it is involved in the cytotoxic response of inflammation and in cell downregulation. Moreover, this mediator prevents the production of pro-inflammatory cytokines, including TNF-α and IL-8[44]. Some studies have demonstrated that SNPs, particularly IL-10-592 (rs1800872) and IL-10-1082 (rs3021097) alleles, might modulate transcriptional activation and affect IL-10 production in vitro. These IL-10 polymorphisms are related to lower mRNA expression of this cytokine and it have been associated with GC development in Asians[45-48].

TNF-α

TNF-α composes the TNF/TNFR cytokine superfamily and it is involved in maintenance and homeostasis of the immune system and host defense[49]. However, this cytokine is related to various pathologic processes, including autoimmunity, chronic inflammatory processes and malignant disease[50]. According to studies, TNF-α signaling through TNFR1 (TNF-α receptor) is important for gastric tumor development[51,52].

Some SNPs in the TNF-α gene are related to increased expression of this cytokine. Among these polymorphisms, TNF-α-857 C/T (rs1799724), TNF-α-308 G/A (rs1800629) and TNF-α-238 G/A (rs361525) are the most studied ones. TNF-α-308 G/A was significantly associated with GC only in Caucasians, while TNF-α-857 and TNF-α-238 were related to an increased risk of gastric tumorigenesis in Asians, but not in Caucasians[53-55].

H. PYLORI VIRULENCE FACTORS AND CARCINOGENESIS

The capacity of *H. pylori* bacteria to trigger a carcinogenic process is not limited to the intense immune response that they unleash, but it also depends on various bacterial factors that can start and modulate neoplastic processes[56]. Different virulent factors found in distinct bacterial strains have been closely associated with the emergence of gastric carcinogenesis. However, genetic variations in genes that encode these virulence factors as well as geographic differences can influence the role of these proteins in GC emergence[15].

CagA

CagA is encoded by the *cagA* gene, present in a DNA

segment containing 30 genes called cag pathogenicity island (*cag PAI*). Infections by strains containing CagA are more capable to induce carcinogenic processes, mainly those with EPIYA-D or more than one EPIYA-C segment[57]. Various *cag PAI* genes are involved in the codification of elements of a pilus structure named type Ⅳ secretion system (TFSS), which has the function of transporting CagA from bacterium to the cytoplasm of the cells from gastric epithelium[58].

After being injected into host cells by TFSS, CagA suffers tyrosine phosphorylation at a carboxi-terminal segment compound by distinct number of EPIYA (Glu-Pro-Ile-Tyr-Ala) regions. There are different EPIYA segments -A, B, C and D-, which contain distinct amino acids in their structure[20]. EPIYA A and B segments are present in most CagA proteins and are followed either by 0-3 EPIYA-C segments in *H. pylori* strains from Occidental countries or by EPIYA-D segments in Eastern countries[59].

Following EPIYA-C or EPIYA-D phosphorylation, an interaction between these segments and SHP-2 possessing SH2 domain occurs, unleashing SHP-2/mitogen-activated protein kinases (MAPK), ERK1, 2-JAK and STAT3 pathways[20]. Cytotoxin associated antigen containing EPIYA-D or more than one EPIYA-C segment ties to SHP-2 more strongly, being more effective in the activation of the pathways mentioned above[60]. This process, activated by CagA, leads to dysfunction of cell growth and of cell-to-cell contact inhibition, cell migration, epithelial cell elongation, and increase of epithelial cell turnover, increasing the propensity of acquirement of precancerous genetic changes by damaged cells[61]. Furthermore, it was demonstrated that relatives of GC patients are more often infected by *H. pylori* strains with more than one EPIYA-C segment in CagA structure[62]. Another study carried out by this same group, performed in a Brazilian population, showed that the host signal transducer and activator of transcription protein 3 (STAT3) rs7744166 polymorphism, as well as being infected by *H. pylori* with CagA containing more than one EPIYA-C segment, are independent predisposing factors for GC[20].

VacA

VacA is another determinant virulence factor in *H. pylori* infection and in gastric carcinogenesis. Patients infected with VacA-positive *H. pylori* strains have a higher propensity for GC development when compared with patients colonized by VacA-negative strains, either in American or in Asian people[63]. Particularly, individuals infected with *H. pylori* strains VacA s1, m1 and s1m1 had an increased risk for gastric carcinogenic unleash in Middle East, Africa and Latin America populations[64]. The peptide mentioned above has only two functional domains in its structure. One of them, the p55-58 domain, has the function of binding to receptors of gastric epithelial cells. The other functional domain, p33-37, produces the cytotoxic effect[65].

VacA is a 90 kDa exotoxin that is activated in a low pH environment[66]. This toxin promotes the generation of numerous acidic vacuoles in gastric epithelial cell cytoplasm[67]. In this process, VacA affects the structure and function of the membrane, the endoplasmic reticulum, the Golgi apparatus and the mitochondria, which can lead to cell death. Furthermore, vacuolating cytotoxin also plays an important role in the activation and suppression of the immune response[68]. This peptide induces a powerful inhibition over T lymphocyte proliferation by means of an interaction with dendritic cells, which are reprogrammed to a tolerogenic genotype[69]. The damage and the immunomodulation performed by this toxin contributes to the increase of gastric mucosa inflammation, ulceration and carcinogenesis in mammals[68].

DupA

Unlike the other virulence factors mentioned in this article, DupA seems to be a protective condition for GC. The *dupA* gene is constituted by two homologue genes of *virB4*, *jhp0917* and *jhp091*, which constitute a continuous gene. The real function of the protein encoded by dupA is still obscure, however, its mechanisms seems to be related to the increase of the production of IL-8 in the gastric antrum, contributing to the development of gastritis that predominates in that gastric region, a process that leads to duodenal ulcer formation[70]. DupA has been significantly associated with duodenal ulcer formation in Asian countries, but this relation was not observed in the Western population[71]. Furthermore, DupA-positive *H. pylori* has been associated with eradication failure[72].

OipA

OipA constitutes a group of peptides described as outer membrane proteins (OMPs), a *H. pylori* protein family composed of 32 components[73]. OipA has been described as a better marker for severe clinical outcomes than CagA, since the infection by strains possessing OipA is an independent determinant risk factor of GC *vs* gastritis in Americans[74,75]. OipA enhances IL-8 production and leads to an increased inflammation status of gastric epithelium. Moreover, it was observed that OipA could inhibit the maturation of dendritic cells in vitro, which might contribute to the immunomodulatory processes performed by *H. pylori*[76].

BabA

babA is a gene that encodes an adhesin whom allows the specific binding to the b and H-1 Lewis antigens, which are expressed in the surface of the gastric mucosa cells[77]. The adhesion of the *H. pylori* to the gastric epithelium mediated by blood group antigen binding adhesin (BabA) appears to play a critical function in the transference of bacterial virulence factors to the host cells. This process contributes to the development of tissue lesions, and a high correlation

of *babA*-positive strains of *H. pylori* with GC has been described[78,79].

CONCLUSION

Despite the wide knowledge about host and *H. pylori* interaction developed since the discovery of its colonization in human stomach, many characteristics that contribute to the infection outcomes are still obscure. The understandings about host polymorphisms in genes that encode cytokines and bacterium virulence factors in GC development are important not only for the determination of patients' prognosis, but it is also a potential way for the development of new preventive and therapeutic strategies.

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P- Reviewer: Barreto SG, Lee CL, Sun X **S- Editor**: Ji FF
L- Editor: Filipodia **E- Editor**: Tan WW

World Journal of Clinical Oncology

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World J Clin Oncol 2018 September 14; 9(5): 90-97

DOI: 10.5306/wjco.v9.i5.90

ISSN 2218-4333 (online)

MINIREVIEWS

Resistance to FLT3 inhibitors in acute myeloid leukemia: Molecular mechanisms and resensitizing strategies

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Author contributions: Zhou J and Chng WJ reviewed the literature and wrote the manuscript.

Supported by the Singapore National Research Foundation and the Ministry of Education under the Research Center of Excellence Program (to Chng WJ); and NMRC Clinician-Scientist IRG Grant, No. CNIG11nov38 (to Zhou J); Chng WJ is also supported by NMRC Clinician Scientist Investigator award; partially supported by the RNA Biology Center at CSI Singapore, NUS, from funding by the Singapore Ministry of Education's Tier 3 grants, No. MOE2014-T3-1-006.

Conflict-of-interest statement: None.

Manuscript source: Invited manuscript

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Received: June 11, 2018
Peer-review started: June 12, 2018
First decision: July 19, 2018
Revised: August 2, 2018
Accepted: August 6, 2018
Article in press: August 7, 2018
Published online: September 14, 2018

Abstract

FMS-like tyrosine kinase 3 (FLT3) is classified as a type Ⅲ receptor tyrosine kinase, which exerts a key role in regulation of normal hematopoiesis. *FLT3* mutation is the most common genetic mutation in acute myeloid leukemia (AML) and represents an attractive therapeutic target. Targeted therapy with FLT3 inhibitors in AML shows modest promising results in current ongoing clinical trials suggesting the complexity of FLT3 targeting in therapeutics. Importantly, resistance to FLT3 inhibitors may explain the lack of overwhelming response and could obstruct the successful treatment for AML. Here, we summarize the molecular mechanisms of primary resistance and acquired resistance to FLT3 inhibitors and discuss the strategies to circumvent the emergency of drug resistance and to develop novel treatment intervention.

Key words: FMS-like tyrosine kinase 3; Tyrosine kinase domain; Internal tandem duplication; FLT3 inhibitor; Drug resistance; Acute myeloid leukemia; Combination therapy

Core tip: FMS-like tyrosine kinase 3 (FLT3) mutations including internal tandem duplication (ITD) or point mutation in tyrosine kinase domain are common genetic

abnormalities in acute myeloid leukemia (AML), predicting dismal outcome. The Federal Drug Administration granted the use of Midostaurin (Novartis) in newly diagnosed FLT3-ITD positive AML in April 2017. A number of other FLT3 inhibitors are in different phases of clinical trials. However, emerging drug resistance poses a major challenge for clinicians to use FLT3 inhibitors. In this manuscript, we systematically reviewed mechanism of primary resistance and acquired resistance to FLT3 inhibitors. We then propose different strategies to overcome drug resistance and novel treatment options for FLT3-ITD positive AML.

Zhou J, Chng WJ. Resistance to FLT3 inhibitors in acute myeloid leukemia: Molecular mechanisms and resensitizing strategies. *World J Clin Oncol* 2018; 9(5): 90-97 Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/90.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.90

INTRODUCTION

Acute myeloid leukemia (AML) consists of a group of different disease characterized with diverse cellular morphologies and various genetic abnormalities[1-5]. Many of these genetic lesions are of clinical importance because they not only implicate in the pathology of AML, but also have prognostic values[6-8]. Mutation in in FMS-like tyrosine kinase 3 (FLT3) confers inferior response to chemotherapy and poor overall survival in AML patients[9-11]. Since the discovery of FLT3 mutations in 1996[12], intensive research effort has provided a better understanding of the molecular mechanism of normal and aberrant FLT3 signaling transduction pathways. Internal tandem duplications (ITDs) in the juxtamembrane domain and activating point mutations in the second tyrosine kinase domain (TKD) occur in near 30% and 10% of patients with AML respectively[13-15].

FLT3 mutations constitutively activate PI3K-AKT, RAS-MEK-MAPK, and STAT5 pathways and result in uncontrolled cell proliferation and cell survival[16-19]. On the other hand, FLT3 mutations suppress myeloid transcription factors PU.1, CCAAT/enhancer-binding protein α (C/EBPα), which result in blocking of myeloid differentiation[20,21]. Thus, FLT3 mutations exert a key role in the pathology of AML, and have been validated as promising intervening targets[22-25]. Currently, Midostaurin (PKC412, Novartis) has been granted by the Federal Drug Administration (FDA) in the use in newly diagnosed FLT3-ITD positive AML in combination with chemotherapy[26]. Moreover, there are about a dozen of other FLT3 inhibitors in different phases of clinical development[27]. Despite most FLT3 inhibitors display strong effectiveness in cell culture system, most of AML patients in trials haven't achieved durable response[28-30]. Notably, AML patients inevitably don't respond to these drugs when they are administrated as single agent for a period. Scientists first observed this resistance phenomenon in patients with chronic myeloid leukemia (CML) who received imatinib mesylate (Gleevec), the first small molecule kinase inhibitor targeting BCR-ABL fusion protein[31].

Here we review published literature on preclinical and clinical findings and molecular mechanisms of primary resistance and acquired resistance to FLT3 inhibitors. We further discuss the strategies to circumvent the emergency of drug resistance and development of novel treatment intervention.

PRIMARY RESISTANCE TO FLT3 INHIBITORS

The identification of a number of de novo and secondary point mutations in the BCR-ABL kinase domain from imatinib-resistant patients promotes researchers to investigate variable sensitivity of FLT3 inhibitors between different activating point mutations in the kinase domain of FLT3.

Based on the mutations identified in AML cases, Grundler *et al*[32] employed site-directed mutagenesis method to create Asp835Tyr, Ile836del and Ile836Met + Arg (numbering is based on the human FLT3) into the cDNA of murine wild-type FLT3. These vectors were then transfected into murine Ba/F3 and 32Dcl3 cells, rendering them independent from growth factors. Tyrphostin AG1296 does not inhibit FLT3 Asp835Tyr (D835Y)-induced proliferation, inhibition of apoptosis, as well as the downstream signaling, and phosphorylation of STAT5. AG1296 is effective on the inhibition of signaling from FLT3 Ile836del (I836del), -ITD and to the less extent, from Ile836Met + Arg (I836M + R). Staurosporin derivative PKC412 is sensitive to all the 3 catalytic domain mutations, but less sensitivity to - *ITD* mutant. Indolinone compound SU5416 shows similar inhibition profile as PKC412. This study suggests that different inhibitors exert a divergent sensitivity toward different mutations in the FLT3 receptors.

A similar approach was used to introduce each FLT3 activation loop mutant, including D835Y, Asp835Ala (D835A), Asp835Glu (D835E), Asp835Gly (D835G), Asp835His (D835H), Asp835Asn (D835N), Asp835Val (D835V), and D835del into human FLT3 cDNA[33]. Ba/F3 cells were transformed with each vector. These 8 activation loop mutations display variable sensitivity toward quinazoline-based inhibitor MLN518 with more than a 10-fold range. I836del is as sensitive as ITD with IC_{50} 0.55 μmol/L. The IC_{50} of D835E, D835A, D835N, D835H ranges from 0.99 to 2.65 μmol/L. D835del, D835V and D835Y confer relative resistance to MLN518 with much higher IC_{50} up to greater than 10 μmol/L.

This phenomenon could be explained by the assumption that the mutations in the amino acid sequence change the conformation of the catalytic domain of FLT3, resulting in a weaken affinity with FLT3 inhibitors[32,33]. However, the structural analysis of these inhibitors in the context of various mutants is not available in these

papers. These findings are of great clinical interesting. Patients enrolled in the FLT3 inhibitor trials potentially can be screened for all known activation loop mutations. In addition, sensitivity of specific inhibitor can potentially be evaluated *ex vivo* prior to clinical administration to avoid known primary resistant cases.

About 1% to 2% of newly diagnosed AML patients carry both ITD and TKD (FLT3-ITD-TKD) with worse outcome when compared with patients with either ITD or TKD mutation alone[34-36]. Similarly, an in vitro study using Ba/F3 cells transfected with FLT3-ITD-TKD dual mutants, for example ITD-D835N and ITD-D835Y, can induce resistance toward not only FLT3 inhibitor SU5614, but also cytotoxic drug Daunorubicin[37]. Molecular study reveals these dual mutants promote overactivation of STAT5 pathway, and result in upregulation of downstream target Bcl-xL and RAD51 and arrest in the G_2/M phase of the cell cycle[37]. Overexpression of Bcl-2 is also detected in primary AML patient samples with FLT3-ITD-Y591 duplication, correlated to high levels of phosphorylated p53. However, whether this mutant induces resistance to FLT3 inhibitors has not been tested[38].

Other possible mechanisms of primary resistance to TKIs have been investigated. P-glycoprotein (p-gp, also named multi-drug resistance 1, MDR1), a major membrane efflux pump, Primary AML blasts co-expressing p-gp and FLT3-ITD, are resistant to herbimycin A, a tyrosine kinase inhibitor, and AG1296, but not to PKC412[39]. The difference could be due to the fact that PKC421 has dually inhibitory roles in FLT3 and protein kinase C (PKC), which can induce phosphorylation of p-gp, resulting in subversion of p-gp mediated MDR. However, other study shows no association between FLT3 mutations and high levels of MDR1 gene expression in AML patients[40].

In contrast to earlier studies, Siendones *et al*[41] demonstrate that inhibition of FLT3-ITD activity does not necessary block the phosphorylation of AKT, ERK and STAT5, which are the 3 major pathways activated by FLT3 mutations, in some primary AML cells. This could be one reason for the limited anti-tumor effect of FLT3 inhibitors used as monotherapy in clinical trials. In addition, a new "niche and leukemia stem cell" model was proposed to explain the limitation of single agent[42]. If FLT3 - ITD is presented in CD34 + CD38 - CD123 + leukemia stem and progenitor cells (LSPC) from primary AML samples, they are more resistant to FLT3 inhibitor in culture under defined nice conditions (fibronectin, IL - 3, SCF, IL - 6 and Ang-). This result is consistent with an earlier finding that patients whose CD34+CD33- precursors harbor FLT3 - ITD have worse outcome than patients whose CD34 + CD33 + progenitors have FLT3 - ITD[43]. These data indicate that FLT3 - ITD AML derived from the less mature progenitors may be associated with drug resistance.

ACQUIRED (SECONDARY) RESISTANCE TO FLT3 INHIBITORS

Pioneer researches in imatinib-resistant CML patients revealed two different resistant mechanisms including increased copy number of BCR-ABL fusion and point mutations in its adenosine triphosphate (ATP) binding motif[44]. These initial discoveries facilitate our understanding of acquired resistance to FLT3 inhibitors. As demonstrated by imatinib-resistant CML studies, over expression of a mutated FLT3 could also be a common mechanism for drug desensitization and leading to resistance. Weisberg and Boulton *et al*[45] first address this issue using a Ba / F3 - FLT3 - ITD resistant polyclonal subline developed by coculture with increasing concentration of PKC412 (up to 40 nmol/L) with the parental cell line over 2 mo. The protein level of FLT3-ITD is significantly increased in this resistant subline compared to the parental Ba / F3 - FLT3 - ITD, leading to desensitize PKC412. It is not clear that FLT3 - ITD protein over expression was regulated on transcriptional (gene amplification) or post-translational levels (increased protein stability). Also, there is no further study on whether this resistant subline harbors point mutation(s) in the TKD domain.

Other resistant lines, designated as Ba / F3 - ITD - R1 to R4 derived from the same parental Ba / F3 - FLT3 - ITD have been developed in the presence of escalatory dose of SU5614[46]. The average IC_{50} of these lines is 17 - fold higher than the parent line. Consistent with their resistant phenotypes, on the molecular level, the phosphorylation of MAPK and STAT5 in these sublines is not inhibited by higher dose up to 10 μmol/L SU5614, while 1 μmol/L of SU5614 effectively decreases activity of MAPK and STAT5 in the parental line. They are also completely resistant to AG1295, which is structurally similar to SU5614. But, Ba / F3 - ITD - R1 to R4 display a similar sensitivity to a structural unrelated FLT3 inhibitor PKC412, a general TKI, Genistein and a chemotherapeutic agent cytosine arabinoside (Ara-C) as the parent ITD cells. Both flow cytometric analysis and western blot demonstrate elevated amount of FLT3 receptor in the resistant Ba / F3 - ITD - R1 to R4 compared with the parent line. Sequence analysis of TKD domain identifies Y842H mutation in ITD-R1 and –R2 cells, and D835 mutation in ITD-R3 and -R4 cells. These data indicate that both FLT3 target desensitization and acquired mutations in the activation kinase domain can contribute to secondary resistance *in vitro*.

Using random PCR mutagenesis to introduce point mutations in ATP-binding pocket of the KD of MSCV-FLT3-ITD, Cools *et al*[47] identified 4 different point mutations (Ala627, Asn676, Phe691, or Gly697) in Ba/F3 cells that render resistance to PKC412, SU5614 or K-252a. The G697R mutation is the most resistant clone to all the three inhibitors tested. Accordingly, PCK412 fails to reduce phosphorylation of FLT3 up to 400 nmol/L in cells with G697R and they are cross-resistant to other structurally different FLT3 inhibitors (GTP-14546, AGL2043, D-64406, D-64476, TMPPP and DQPPC). Modeling crystal structure of FLT3 receptor in complex with PCK412 indicates that the amino acid Gly697 and Phe691 directly contact with PKC412 and substitution

Table 1 Summary of main studies on primary and acquired resistance to FMS-like tyrosine kinase 3 inhibitors

	Ref.	Disease model (method and material)	Mechanisms of resistance
Primary resistance	Grundler *et al*[32], 2003	Site-directed mutagenesis, murine Ba/F3	TKD mutation, deletion or insertion
	Clark *et al*[33], 2004	Site-directed mutagenesis, murine Ba/F3	TKD mutation
	Bagrintseva *et al*[37], 2005	Site-directed mutagenesis, murine Ba/F3	ITD-TKD mutation, Bcl-xL overexpression
Acquired resistance	Weisberg *et al*[45], 2002	Coculture with PKC412, murine Ba/F3-FLT3-ITD	FLT3 protein overexpression
	Bagrintseva *et al*[46], 2004	Coculture with SU5614, murine Ba/F3-FLT3-ITD	ITD-TKD mutation, FLT3 protein overexpression
	Cools *et al*[47], 2004	Random PCR mutagenesis, murine Ba/F3-FLT3-ITD	ITD-TKD mutation
	Heidel *et al*[48], 2006	PKC412 clinical trial, relapsed AML with ITD	ITD-TKD (N676K) mutation
	Piloto *et al*[49], 2007	Coculture with CEP-5214 and CEP-701, human MOLM-14, Hb1119 and SEM-K2	RTK amplification, N-Ras mutation
	Zhou *et al*[50], 2009	Coculture with ABT-869, human MV4-11	Overactivation of STAT, overexpression of Survivin

FLT3: FMS-like tyrosine kinase 3; ITD: Internal tandem duplication; TKD: Tyrosine kinase domain; RTK: Receptor tyrosine kinase; STAT: Signal transducer and activator of transcription.

Gly697 with a larger amino acid will decrease its binding affinity due to possible steric clash with the FLT3 inhibitors. Importantly, mutation in Asn676 (N676K) has been reported in 1 of 6 patients with FLT3-ITD AML who relapsed after PKC412 treatment in a phase 2 clinical trial[48]. The authors were able to rule out other common mechanisms of drug resistance including gene amplification, overexpression of FLT3 protein, drug metabolism, drug efflux, inhibition by serum proteins and major deficiency in apoptosis pathway. Although the identification of N676K is clinically significant to elucidate the mechanism of resistance and relapse, so far acquired point mutation of TKD has been reported only in a FLT3 inhibitor treated, and relapsed AML patient. In addition, transfection of FLT3-ITD-N676K in 32D cells confers resistance to PKC412[48]. This finding is in consistent with the clinical observation that this mutant could be the sole reason of secondary resistance.

Most of the initial pre-clinical studies on mutations were conducted in murine cell lines transfected with FLT3 cDNA[32,33,37,45-47]. We and others to have further investigated the molecular mechanisms of acquired resistance to FLT3 inhibitors. Human leukemia cell lines with FLT3 mutations are valuable and relevant models for molecular biology and drug sensitivity studies. MV4-11 and MOLM-14 cell lines were derived from primary AML cells, while MV4-11 has two FLT3-ITD alleles; MOLM-14 harbors one mutant FLT3-ITD allele, while the other allele is wild-type (WT). Leukemia cell line Hb1119 and SEM-K2 were derived from primary ALL (acute lymphoblastic leukemia) cells. Hb1119 harbors FLT3-D836H, whereas SEM-K2 over expresses wild-type FLT3. Piloto *et al*[49] reported that prolonged coculture of MOLM-14, Hb1119 and SEM-K2 cells with CEP-5214 and CEP-701 respectively leaded to the development of resistant lines including M14(R)5214, M14(R)701, Hb(R)5214, Hb(R)701, SEM(R)5214 and SEM(R)701. They are cross resistant to PKC412 and AG1295, a structurally related FLT3 inhibitor[49]. Although TKIs can inhibit phosphorylation of FLT3 receptor in most of the resistant clones as demonstrated in this study, the downstream Akt and/or MAPK signaling remain activated, thus providing cells sustained survival and proliferative signaling. Acquired N-Ras mutations have been identified in 2 [M14 (R) 5214 and M14 (R) 701] out of the 6 resistant lines. Transducing N-Ras-G12V mutation into MOLM-14 cells results in resistance to CEP-701[49]. So, activation of parallel signaling pathway independent to FLT3 signaling may contribute to secondary resistance in some cases.

Through long-term culture of MV4-11 cell line with the FLT3 inhibitor, ABT-860, a FLT3 inhibitor-resistant line, MV4-11-R was generated[50]. The IC_{50} of ABT-869 for MV4-11-R line is 52 nmol/L *vs* 6 nmol/L for the parental MV4-11 cell line. Importantly, other structurally unrelated inhibitors including SU5416, AG1296 and a FLT3 inhibitor Ⅲ from MERCK, were not effective to MV4-11-R line anymore, suggesting a cross resistant circumstance. Sequencing analysis showed normal sequence of FLT3-TKD in MV4-11-R cells. Western blot and FACS analysis excluded the overexpression of p-FLT3, FLT3 and three multidrug resistance related proteins (MDR, MRP1 and LRP) in this resistant line. But, overexpression of FLT3LG and Survivin was demonstrated at the both transcript and protein level. Down-regulation of suppressor of cytokine signaling (SOCS) proteins (negative regulators of STAT pathways) was also observed in the presence of overactivation of the STAT1, STAT3 and STAT5 pathways in this resistant line. In conclusion, our findings show that overactivation of STAT pathways and subsequently increased expressions of surviving genes are the main mechanism of resistance to FLT3 inhibitors. A total of 9 main studies regarding to primary and acquired resistance is summarized in Table 1.

STRATEGIES TO CONQUER RESISTANCE

The understanding of molecular mechanisms of primary and secondary resistance to FLT3 inhibitors (Figure 1) provides the foundation for establishing strategies to conquer or reduce resistance. Combination of FLT3 inhibitors with cytotoxic drugs or other small molecule inhibitors targeting different pathways has been extensively searched and tested *in vitro*, in murine

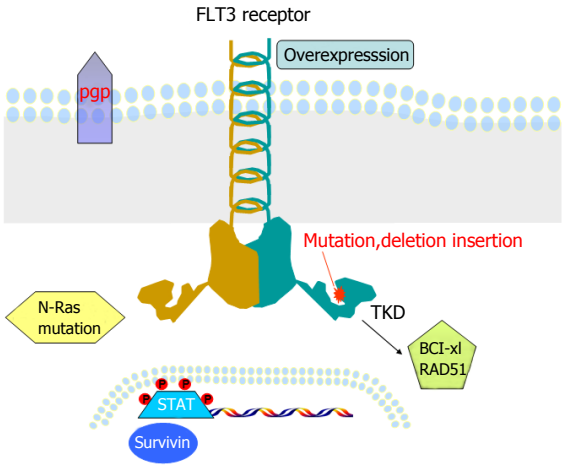

Figure 1 Schematic representation of various published mechanisms of resistance to FMS-like tyrosine kinase 3 inhibitors. This figure shows a number of models of resistance including desensitization of drug targets by *FLT3* gene amplification or protein overexpression, decreased drug binding affinity by mutation, deletion or insertion in TKD, increased drug efflux by p-gp, activation of survival and proliferative pathways (molecules) such as RAS pathway, STAT pathway, anti-apoptotic Bcl-xL, Survivin and DNA repair molecule RAD51. FLT3: FMS-like tyrosine kinase 3; TKD: Tyrosine kinase domain; STAT: Signal transducer and activator of transcription.

xenograft models and some in clinical trials.

We and other investigators have demonstrated that combination of FLT3 inhibitors with conventional chemotherapy drugs, such as cytarabine and doxorubicin, can achieve synergistic effect[30,51-54]. The optimal combination sequence should start with chemotherapy, followed by FLT3 inhibitors to maximize synergism and potential to reduce and/or overcome resistance[30,53].

Combination of FLT3 inhibitors with a spectrum of small molecules inhibitors targeting downstream or independent signaling pathways have been evaluated in pre-clinical studies and showed early promises. Rapamycin, an mTOR inhibitor, sensitizes not only Imatinib-resistant BCR-ABL positive cells[55] but also TKI-resistant Ba/FLT3 dual mutant (ITD and TKD) cell[46,55]. Approaches targeting cellular apoptosis machinery also have been explored. BH3 mimetic ABT-737, a potent inhibitor of anti-apoptotic Bcl2, effectively neutralizes resistance to FLT3 inhibitors in primary AML blasts[56]. The proapoptotic inhibitor LBW242, a Smac (one member of the inhibitor of apoptosis, IAP) mimetic, can overcome resistance to PKC412 when used in combination with PKC412[57]. Combination of FLT3 inhibitor GTP14564 with a HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17-AAG), produces synergism via STAT5 pathway[58]. Concurrent treatment with histone deacetylase inhibitor (HDACi) LAQ824 and PCK412 can synergistically induced apoptosis in human cell line and primary AML samples with FLT3 mutations[59]. We demonstrate that either treatment with IDR E804, an inhibitor of CDKs and the SRC-STAT pathway, or targeting Survivin by shRNA or a dominant-negative vector (survivin-T34A) sensitize MV4-11-R to ABT-869 induce apoptosis[50].

Other compounds such as bis(1*H*-indol-2-yl) methanone Cpd.98, Cpd.102 and Sorafenib (B-Raf inhibitor) also overcome resistance to FLT3 inhibitors[60,61]. Downregulation of FLT3 expression by RNAi increases sensitivity to FLT3 inhibitor MLN518 in human AML cell lines, a potential approach to override resistance[62]. The PIM family of serine/threonine kinases (PIM-1, -2 and -3) has been shown to be cytoprotective[63]. Constitutively activated FLT3 signaling up-regulates the PIM-1 expression via STAT5 pathway, which results in phosphorylation of BAD protein (pSer112 and pSer136), exerting anti-apoptotic effect[63,64]. PIM-2 also phosphorylates BAD at Ser-112, blocking BAD-inducing cell death[65]. Silencing PIM-2 or PIM-1 sensitizes resistant cells to FLT3 inhibitors[66]. IMC-EB10, an anti-FLT3 monoclonal antibody, is still effective in FLT3-TKI resistant clones, because it mediates antibody-dependent, cell-mediated cytotoxicity (ADCC) which is independent of the FLT3-ITD signaling pathway[49].

CONCLUSION

Primary and secondary resistance to TKI therapy is challenging issue in modern anti-cancer warfare for various cancers including AML. At present time, monotherapy using FLT3 inhibitors showed limited benefit in relapsed AML clinical trials. We now began to better understand the molecular mechanisms of resistance in FLT3 targeting. Ongoing early phase clinical trials are important to further shed light on various potential mechanisms of resistance, and will eventually facilitate better strategies to prevent and overcome resistance. Sequel combination of FLT3 inhibitors with chemotherapy or other small molecule inhibitors targeting mTOR, HDAC, HSP90, STAT3, Bcl2, PIM family, IAPs (Survivin and Smac) and others are ongoing strategies. The correlative studies with these ongoing trials for identifying resistance mechanisms among trial patients, will help investigators in refining the design for next generation trial protocols. In addition, by determining "oncogenic signature" of each patient prior to treatment should guide the proper choice of most efficient combinations targeting the specific "oncogenic signature" individually.

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P- Reviewer: Chiang TA, Gadbail AR, Ghorbian S, Morelli F
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Wu YXJ

World Journal of Clinical Oncology

Submit a Manuscript: http://www.f6publishing.com

World J Clin Oncol 2018 September 14; 9(5): 98-109

DOI: 10.5306/wjco.v9.i5.98

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Tunable structure priors for Bayesian rule learning for knowledge integrated biomarker discovery

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Author contributions: Balasubramanian JB developed the concept, conducted the research, and prepared the first draft of the manuscript in consultation with research mentor and senior author Gopalakrishnan V; All authors contributed to writing and editing the manuscript.

Supported by National Institute of General Medical Sciences of the National Institutes of Health, No. R01GM100387.

Conflict-of-interest statement: The authors declare no conflicts of interest with respect to the submitted manuscript.

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Received: April 27, 2018
Peer-review started: April 27, 2018
First decision: July 9, 2018
Revised: July 24, 2018
Accepted: August 5, 2018
Article in press: August 5, 2018
Published online: September 14, 2018

Abstract

AIM

To develop a framework to incorporate background domain knowledge into classification rule learning for knowledge discovery in biomedicine.

METHODS

Bayesian rule learning (BRL) is a rule-based classifier that uses a greedy best-first search over a space of Bayesian belief-networks (BN) to find the optimal BN to explain the input dataset, and then infers classification rules from this BN. BRL uses a Bayesian score to evaluate the quality of BNs. In this paper, we extended the Bayesian score to include informative structure priors, which encodes our prior domain knowledge about the dataset. We call this extension of BRL as BRL$_p$. The structure prior has a λ hyperparameter that allows the user to tune the degree of incorporation of the prior knowledge in the model learning process. We studied the effect of λ on model learning using a simulated dataset and a real-world lung cancer prognostic biomarker dataset, by measuring the degree of incorporation of our specified prior knowledge. We also monitored its effect on the model predictive performance. Finally, we compared BRL$_p$ to other state-of-the-art classifiers commonly used in biomedicine.

RESULTS

We evaluated the degree of incorporation of prior knowledge into BRL$_p$, with simulated data by measuring the Graph Edit Distance between the true data-generating model and the model learned by BRL$_p$. We specified the true model using informative structure

priors. We observed that by increasing the value of λ we were able to increase the influence of the specified structure priors on model learning. A large value of λ of BRL_p caused it to return the true model. This also led to a gain in predictive performance measured by area under the receiver operator characteristic curve (AUC). We then obtained a publicly available real-world lung cancer prognostic biomarker dataset and specified a known biomarker from literature [the epidermal growth factor receptor (*EGFR*) gene]. We again observed that larger values of λ led to an increased incorporation of EGFR into the final BRL_p model. This relevant background knowledge also led to a gain in AUC.

CONCLUSION

BRL_p enables tunable structure priors to be incorporated during Bayesian classification rule learning that integrates data and knowledge as demonstrated using lung cancer biomarker data.

Key words: Supervised machine learning; Rule-based models; Bayesian methods; Background knowledge; Informative priors; Biomarker discovery

Core tip: Bayesian rule learning is a unique rule learning algorithm that infers rule models from searched Bayesian networks. We extended it to allow the incorporation of prior domain knowledge using a mathematically robust Bayesian framework with structure priors. The hyperparameter of the structure priors enables the user to control the influence of their specified prior knowledge. This opens up many possibilities including incorporating uncertain knowledge that can interact with data accordingly during inference.

Balasubramanian JB, Gopalakrishnan V. Tunable structure priors for Bayesian rule learning for knowledge integrated biomarker discovery. *World J Clin Oncol* 2018; 9(5): 98-109 Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/98.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.98

INTRODUCTION

Knowledge discovery from databases (KDD) is the non-trivial extraction of valid novel, potentially useful, and understandable patterns from the dataset[1]. Data mining is the computational process of the extraction of these patterns. In biomedicine, data mining is extensively applied for knowledge discovery[2]. The recent advances in biomedical research, triggering an explosion of data, have encouraged these applications. Particularly, the development of high-throughput "omic" technologies has generated a large number of datasets, which provide a holistic view of a biological process. These datasets present opportunities to discover new knowledge in the domain. They also present some challenges, especially from their high-dimensionality. High-dimensional datasets are challenging to data mining algorithms because several thousands of candidate variables (*e.g.,* gene expressions or SNPs) can potentially explain an outcome variable of interest (*e.g.,* phenotypes or disease states) but have only a few instances as evidence to support an explanation. These large numbers of candidate variables generate a model search space that is very large for data mining algorithms to explore efficiently, and having only a few instances generates uncertainty for the algorithm to determine the correctness of any candidate model. In such model search spaces, data mining algorithms can easily get stuck in local optima or they may infer associations between spurious variables and the outcome variable, by chance.

Fayyad *et al*[3], emphasized the importance of domain prior knowledge in all steps of the KDD process. In biomedicine, often in addition to the dataset, we have some prior domain knowledge about the dataset. This domain knowledge can help guide the data mining algorithm to focus on regions in the model search space that are either objectively more promising for a given problem or subjectively more interesting to a user. The prior knowledge can come from domain literature (*e.g.,* searching through PubMed), a domain expert (*e.g.,* a physician), domain knowledge-bases (*e.g.,* Gene Ontology) or from other related datasets [*e.g.,* from public data repositories like Gene Expression Omnibus (GEO)]. It is now imperative to develop data mining methods that can leverage domain knowledge to assist with the data mining process.

Rule learning methods are among the oldest, well-developed, and widely applied methods in machine learning. They are particularly attractive for KDD tasks because they generate interpretable models with understandable patterns and have good predictive performance. Interpretable models are succinct, human-readable models that explain the reasoning behind their predictions. Bayesian rule learning (BRL) is a rule learning method that has been shown to perform better than state-of-the-art interpretable classifiers on high-dimensional biomedical datasets[4,5]. BRL takes a dataset as input and searches over a space of Bayesian belief-networks (BN) to identify the BN that best explains the input dataset. BRL then infers a rule model from this BN. BRL uses the Bayesian score[6] as a heuristic to evaluate a BN during search. The score allows the user to specify a prior belief distribution over the space of BNs that encodes our prior beliefs about which models are more likely to be correct than others with respect to our domain knowledge. Typically in literature uninformative priors are used, which means that we claim that a priori all models are equally likely to be correct. As we saw earlier, often along with the dataset, additional domain knowledge is available that can assist with the data mining process. These sources lead us to believe that some models are more likely to

be correct than others even before we see the dataset. We can specify this belief using informative priors. Two approaches to using informative priors in literature have shown promise[7,8]. In the Methods and Materials section of this paper, we discuss each of the two approaches and describe ways to extend BRL to specify such informative priors that can incorporate domain knowledge.

In this paper, we implemented an approach to incorporate prior domain knowledge into the BRL learning process using informative priors. We evaluated the effect of this prior knowledge on model learning using experiments with simulated and a real-world lung cancer prognostic dataset.

MATERIALS AND METHODS

In this section, we describe our implementation in BRL to incorporate prior domain knowledge, and then describe two experiments we conducted to evaluate this implementation. Specifically, we describe a BRL greedy best-first search algorithm, the heuristic score used by the search to evaluate candidate models, and our approach to extend this heuristic score to incorporate prior background domain knowledge using informative priors. We call this extension to BRL as BRL$_P$ (BRL with informative priors). After describing our implementation of BRL$_P$, we describe two experiments we conducted to study the effects of informative priors in model learning: (1) using simulated data; and (2) on a real-world lung cancer prognostic dataset.

BRL

BRL is a rule-based classifier that takes as input, a dataset D, and returns a rule set model. Let the dataset D be an observed instantiation of a system with a probability distribution over a set of n random variables and a target random variable of interest,$D = \{X_i,T_i; i \in 1...n\}$. Here, T is the target variable of interest, which is the dependent variable for the prediction task. Every other variable, X_i in D is an independent random variable that may help predict T. There are a total of m instances in D. In the classification problem, our task is to accurately predict the value of the target variable. For example, consider a diagnostic problem of predicting a disease outcome for a patient, say lung cancer outcome (either Case or Normal), using gene expression biomarker data, measured for each patient. Here, the dataset D would be composed of a set of m patients, each with n gene expression measurements $\{X_i; i \in 1...n\}$. The target variable T is the binary-valued lung cancer outcome variable, $T = \{Case, Normal\}$, for each patient in the dataset.

The BRL search algorithm explores a space of BNs, learned from the observed dataset D, and returns the most optimal BN found during the search. A BN is a graphical representation of the probabilistic dependencies of the different variables in the system under study. They are represented as a directed acyclic graph (DAG). In our lung cancer diagnostic problem example, an example of probabilistic dependence could be some hypothetical gene expression, say the binary-valued $X_A = \{Up; Down\}$ with a value for up-regulated and a value for down-regulated gene A, is known to be predictive of the outcome T. Then an optimal BN should contain a directed edge from $X_A \rightarrow T$. In other words, the lung cancer outcome depends upon whether or not gene X_A is expressed. In such a BN, the probability distribution, $P(T \mid X_A)$ is the parameter of the BN.

The parameters of the BN can be represented in form of a conditional probability table (CPT). The CPT is often stored in form of decision trees[9,10]. The BRL generates a mutually exclusive and exhaustive set of inference rules from this decision tree for prediction of class of any new test instances. Here, each path from root to leaf of the decision tree is interpreted as a rule. The BRL rules are represented in the form of explicit propositional logic: IF antecedent THEN consequent. The rule antecedent is the condition made up of conjunctions (ANDing) of the independent random variable-value pairs, which when matched to a test instance, implies the rule consequent composed of the dependent target variable-value. Continuing with our example, a learned rule can be IF ($X_A = Up$) THEN ($T = Case$). In other words, if the gene X_A is up-regulated then the patient is classified to have a lung cancer outcome as a *Case*. There are several types of BRL search algorithms[4,5,11] to help find the optimal BN. In this paper, we will only discuss a simple greedy best-first search algorithm from our previous work[4] and is summarized in the next sub-section.

BRL greedy best-first search algorithm: The BRL greedy best-first search algorithm is described in detail in the paper by Gopalakrishnan *et al*[4], where it is referred to as BRL$_1$. In this paper, we will refer to this algorithm simply as BRL. We will summarize the algorithm in this subsection. The BRL algorithm initializes the search with a network structure with just the variable T and no parent nodes. In each iteration of the algorithm, one new parent is added to T among the n random variables that is not already a parent of T. This BN implies the hypothesis that T is dependent upon the set of variables added as parents to T. This process is called model specialization. The resulting models from that iteration is added to a priority queue. The priority queue sorts these specialized models by evaluating them using a heuristic score called the Bayesian score, which evaluates the likelihood that the observed dataset was generated by a given hypothesized BN model. This score is described in detail in the next subsection. The greedy search picks the model in the head of the priority queue at the end of the iteration. This model is evaluated to be the best scoring model among the specializations in that iteration. In the next iteration, this model is selected for further specialization by adding more parents. The search terminates when a subsequent specialization step fails to improve the

heuristic score. The search also terminates if the model has reached a limit on the maximum number of parents allowed for *T*. This search parameter is called maximum conjuncts. Finally, BRL generates a rule model inferred from the model returned by the search.

BRL heuristic score (Bayesian score): BRL search evaluates the quality of a candidate BN model using a heuristic score called the Bayesian score[9]. In this sub-section, we describe this score. We represent a BN model as the tuple $B = (B_S, B_P)$, where B_S is the network structure with a subset of π discrete-valued nodes, and B_P is the numerical parameters of the network. The posterior probability of the candidate structure given the observed dataset, *D*, is calculated as in Equation 1.

$$P(B_S \mid D) = P(B_S, D)/P(D) \quad (1)$$

Since we are comparing Bayesian networks learned from the same dataset *D*, the denominator does not affect our decision. Only the numerator helps with model selection as shown in Equation 2.

$$P(B_S \mid D) \propto P(B_S, D) \quad (2)$$

The joint probability of the network structure and the observed dataset, $P(B_S, D)$, is equal to the prior probability of the network structure, $P(B_S)$ and the likelihood that the observed data was generated by that network structure,$P(D \mid B_S)$. This is shown in Equation 3.

$$P(B_S, D) = P(B_S) \cdot P(D \mid B_S) \quad (3)$$

To compute the joint probability of the network structure and the observed dataset, $P(B_S, D)$, we use the BDeu score[6]. We get Equation 4.

$$P(B_S, D; \alpha) = P(B_S) \cdot \prod_{i=1}^{n} \prod_{j=1}^{q_i} \frac{\Gamma\left(\frac{\alpha}{q_i}\right)}{\Gamma\left(N_{ij} + \frac{\alpha}{q_i}\right)} \prod_{k=1}^{r_i} \frac{\Gamma\left(N_{ijk} + \frac{\alpha}{r_i q_i}\right)}{\Gamma\left(\frac{\alpha}{r_i q_i}\right)} \quad (4)$$

Here, *i* iterates through each node in the BN with *n* nodes. Index *j* iterates though all, q_i, possible variable-value instantiations of the parents of the i^{th} node. Index *k* iterates through all r_i values of the i^{th} node. N_{ijk} is the number of instances in *D*, where the variable *i* takes the k^{th} value and its parent variables take the j^{th} variable-value instantiation, and $N_{ij} = \sum_k N_{ijk}$. The Gamma function is defined as $\Gamma(x) = (x\text{-}1)!$. The α is a user-defined parameter called prior equivalent sample size (*pess*). We set $\alpha = 1$, which allows the data to easily dominate the score[9]. The $P(B_S)$ term is called the structure prior (see[9] section 18.3.6.1 for details) that represents the prior belief distribution over all network structures before we look at the data. The remaining terms in Equation 4 compose the likelihood term that infers the likelihood of the network from the observed data.

In the classification task using BRL, we do not learn a fully generalized BN but only care about the relationship of the variables with a specific target variable of interest, *T*. Variable *T* is discrete with different values. The set of parents of the i^{th} variable is represented as π_i. In BRL, we learn a constrained BN with node *T* and its set of parents, π_T. The set π_i can have q_T possible attribute-value instantiations. So, for BN search in BRL, we optimize the heuristic score in Equation 5.

$$P(B_S, D) = P(B_S) \cdot \prod_{j=1}^{q_T} \frac{\Gamma\left(\frac{\alpha}{q_T}\right)}{\Gamma\left(N_j + \frac{\alpha}{q_T}\right)} \prod_{k=1}^{r_i} \frac{\Gamma\left(N_{jk} + \frac{\alpha}{r_T q_T}\right)}{\Gamma\left(\frac{\alpha}{r_T q_T}\right)} \quad (5)$$

The expectation of each parameter value of the BN is computed with Equation 6.

$$\mathbb{E}[\theta_{jk} \mid D, B_S] = \frac{N_{jk} + \frac{\alpha}{r_T q_T}}{N_j + \frac{\alpha}{q_T}} \quad (6)$$

We use this value as the posterior probability of the rule. The number of rules inferred by BRL is equal to the number of θ_{jk} values in the BN. The expectation of this value shows the degree of support a rule has in the observed dataset.

BRL with structure priors: In Equation 5, the $P(B_S)$ term is the structure prior that represents the prior distribution over all network structures. Here, we can specify our prior bias of certain network structure over others to skew the BRL search to focus on certain network structures more than others. Typically, in literature uninformative priors are used, which means that a priori we claim that we do not have any preference of network structures over the others. BRL in this case lets the data alone decide the final learned model. The challenge of specifying these priors is that the total number of network structures grows super-exponentially with the number of variables *n*[12]. It often becomes infeasible to specify structure priors for each of these network structures for even moderately sized datasets. So far in BRL, we had been using an uninformative prior by setting $P(B_S) = 1$, in Equation 5.

Castelo and Siebes[7] describe a promising approach to elicit structure priors by specifying the probability of the presence or absence of each edge in the network structure. The user only needs to specify the probability of a subset of edges in the network structure. The probabilities for all the remaining edges are assigned a discrete uniform distribution value. A challenge using this approach is to specify the values of these probabilities. In our experiments with BRL using these priors, we observed that the likelihood term in Equation 5 always dominates the structure prior term. It would help us if we could control the influence of structure priors over the likelihood term using a scaling factor. As we described earlier in the introduction section, the background knowledge, we specify, itself has uncertainty associated with it. A scaling factor would help us control the influence of data and our prior knowledge.

Mukherjee and Speed[8] propose an informative prior that uses a log-linear combination of weighted real-valued function of the network structure, $f_i(B_S)$. This function is called the concordance function. It can be any function that monotonically increases with the increase in agreement between the learned network structure and the prior beliefs of the user. This is shown in Equation 7.

$$P(B_S) \propto exp\left[\lambda \cdot \sum w_i f_i(B_S)\right] \quad (7)$$

The hyperparameter w_i are the positive weights that represent the relative importance of each function. The hyperparameter λ is a scaling factor that helps to

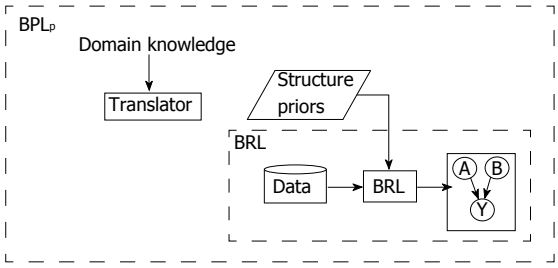

Figure 1 The Bayesian rule learning framework that can incorporate domain knowledge. BRL: Bayesian rule learning.

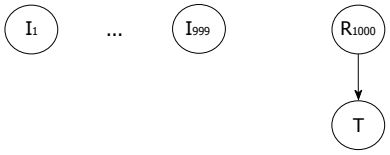

Figure 2 The data-generating graph for the simulated data.

control the overall influence of the structure prior. This will help us quantify the uncertainty in the validity of our prior knowledge.

The structure prior we used for BRLp comes from an instantiation of the general form of this prior, shown in Equation 7, as described by Mukherjee and Speed[8]. It allows the user to specify their prior beliefs about the presence and absence of the edges in the network structure. This instantiation is shown in Equation 8.

$$P(Bs) \propto exp\,[\lambda \cdot (|E(Bs) \cap E_{+}| - |E(Bs) \cap E_{-}|)] \quad (8)$$

Here, set E_{+} (positive edge-set) represents the set of edges the user believes should be present in the model, and set E_{-} (negative edge set) represents the set of edges the user believes should be absent from the model. So, the concordance function in this instantiation simply gives a positive count for if the candidate graph contains an edge from the positive edge-set, and a negative count (penalty) when it contains an edge from the negative edge-set. In this instantiation, the weights hyperparameter is set to 1, since our counts are all valued 1. We need to learn the value of the hyperparameter λ. The range of values it can take depends upon the well-known Jeffrey's scale[13]. When $\lambda = 0$, the whole exponent becomes 0, and $P(Bs) = exp(0) = 1$, which is the uninformative prior. In other words, when $\lambda = 0$, BRLp should have no effect of structure prior and so would behave the same as the baseline model, BRL. As we increase the value of λ, the effect of the structure prior would have an increased influence over the likelihood term in Equation 5.

To summarize, BRLp uses a heuristic score called the BDeu score, shown in Equation 5, and encodes the structure prior in that score using Equation 8. The BRLp framework is shown in Figure 1. The inner dotted box, labeled "BRL", is the classic BRL without prior knowledge, which takes in an input dataset, uses BRL algorithm to learn and output a model. The outer dotted box is our extension, BRLp that can incorporate domain knowledge. The translator process, currently done manually, converts knowledge from various sources to input into Equation 8.

Experiment design

In this section, we describe our experiment design that we used to demonstrate the functionality of BRLp. We examined its behavior on both, simulated dataset, and on a real-world dataset. We were mainly interested in the ability of BRLp to incorporate the supplied prior domain knowledge with respect to the structure prior hyperparameter λ. Additionally, we also monitored the changes in the predictive power of the learned model resulting from the influence of the supplied prior domain knowledge. We studied the functionality of BRLp on a simulated dataset, and then on a real-world dataset. Each is described, in detail, in the following sub-sections.

Simulated data analysis: We first generated simulated data to study the behavior of BRLp. We can control the properties of the simulated dataset, which gave us a controlled environment to check if BRLp was behaving as we expected on a dataset with the specified properties.

Data generation: We generated a simulated dataset with 1000 variables in addition to the target variable, *T*. We show the data-generating graph in Figure 2. Out of the 1000 candidate variables that can predict *T*, only one variable, R_{1000}, is relevant. A relevant variable is a variable that helps to predict *T*. All the remaining 999 variables, $\{I_1...I_{1000}\}$, are irrelevant. Irrelevant variables are random values that do not help predict *T*. All the random variables in the graph are binary {0, 1}. The conditional distributions in the graph are Bernoulli with the success parameter *p* depending upon the value instantiation of their parent variables. The irrelevant and relevant variable values were randomly sampled with $p = 0.5$. The *T* variable value was sampled with $p = 0.9$ if its parent, R_{1000}, took the value 1, and $p = 0.1$ otherwise.

Data background knowledge: In a simulation problem, we already knew the true data-generating graph as shown in Figure 2. We knew that in the learned network structure from BRLp, there should be an edge present between R_{1000} and *T*. So, in Equation 8, the positive edge-set only contained this edge, $E_{+} = \{(R_{1000}, T)\}$. All the edges between irrelevant variables and *T* should be absent in the BRLp model, so they went to the negative edge-set, $E_{+} = \{(I_k, T); k = 1...999\}$. We evaluated the impact of the λ hyperparameter value of the structure prior on the final model learned by BRLp.

Methods evaluated: We evaluated the method BRLp here. We set the user-defined, search algorithm

parameter of BRL$_p$ of maximum conjuncts (constraint on maximum number of parents of *T*) to 8. We evaluated the effect of the hyperparameter λ by assigning =its values λ = {0,1,2,3,4,5,6,7,8,9,10}. The value of λ = 0 represents the baseline model of BRL with no structure priors.

Evaluation metrics: We evaluated BRL$_p$ with two metrics: (1) graph edit distance (GED); and (2) area under the receiver operator characteristics curve (AUC). We evaluated them over 5 runs of 10-fold cross-validation. In each run, the dataset was randomly shuffled to produce a different set of 10 stratified folds. GED measures how much of the prior domain knowledge gets incorporated into the model learning process. Specifically, how much does the model learned by BRL$_p$ agree with the supplied prior knowledge? This metric is described in detail in the next paragraph. We monitored the BRL$_p$ model predictive power by measuring the average AUC across the 5 runs of 10-fold cross-validation. The AUC helped us monitor the influence of structure priors in model predictive performance.

GED[14] is a metric of similarity between two graphs. In this experiment, we compared two constrained BNs. Specifically, we were interested in measuring how closely our BRL$_p$ predicted BN, $\widehat{B}_S$ (learned by BRL$_p$) resembled the true BN, B_S, which generated the simulated dataset (Figure 2 in this experiment). This was used to estimate the value of adding structure prior knowledge for model learning when the true model is available for comparison. We computed this metric using Equation 9.

$$d_{v_{min}}(B_S, \widehat{B}_S) = \min_{v \in \Upsilon(B_S, \widehat{B}_S)} \sum_{e_i \in v} c(e_i) \qquad (9)$$

Here, $d_{v\,min} = [B_S, \widehat{B}_S]$ is a function that returns the GED between the two BNs. A specific e_i is an edit operation to transform one graph into another. For the constrained BN we have two available edit operations - delete edge, and insert edge. There is a cost c (e_i) associated with each edit operation. We set c (e_i) = -1, for both the edit operations. A *v* is an edit path containing a sequence of edit operations to transform graph B_S into $\widehat{B}_S$. The set γ [B_S, $\widehat{B}_S$] is a set of all possible edit paths. To compute the graph edit distance, we find the edit path, *v*, that minimizes the overall cost and then return this minimum cost value indicating the minimum number of operations needed to transform one graph to another. Therefore, an edit distance of 0 indicates that the predicted graph is identical to the true graph. Since the maximum parents resulted from BRL is constrained to 8 from the user parameter, the worst possible model contains all 8 irrelevant variables. So, we get $d_{v\,min} = 9$ (8 edge deletion operations from irrelevant variables, 1 insert edge operation to the relevant variables).

Real-world lung cancer prognostic biomarker data analysis: We obtained a real-world dataset for our analysis from Gene Expression Omnibus[15] (GEO), a public gene-expression data repository. We extracted the dataset from a study[16] that collected both tumor and normal tissue samples from 60 female non-small cell lung cancer (NSCLC) patients in Taiwan. As a result, there were 120 samples in this dataset (60 patients, each with paired tumor and normal tissue). RNA was extracted from these paired tumor and normal tissues for gene expression analysis on the Affymetrix Human Genome U133 Plus 2.0 Array platform. The platform has 54675 probes. The accession ID for this study on GEO database is GSE19804.

Data pre-processing: The raw dataset extracted from GEO contained 54675 probes and 120 instances. We needed to pre-process the data to prepare it for data analysis. The dataset pre-processing was done using Bioconductor (version 3.6) packages in R (version 3.4.3). We extracted the raw dataset using the *affy* package[17]. We used Robust Multichip Analysis (RMA) for background correction, quantile normalization, and probe summarization. We mapped probes to the genes they represented. Multiple probes can map to a single gene. In the final dataset, we would like to have just one random variable representing a unique gene. Among the multiple probes that map to a single gene, we chose the probe with the largest inter-quantile range to represent the gene. This process is called inter-quantile range (IQR) filtering. Finally, we also extracted the tissue phenotype (tumor or normal) for each sample and add to this dataset. The outcome variable of interest was this tissue phenotype. After this pre-processing step, we were left with 16382 genes. So, the final dataset for our analysis had 16382 variables and 120 instances. The R script we used for data pre-processing is available in the GitHub repository linked in the Conclusion section.

Many classification algorithms, including BRL, cannot handle continuous-valued variables, and require the input data to be discretized. Moreover, supervised discretization can help improve the performance of several classifiers including Support Vector Machines and Random Forests[18]. This is because supervised discretization acts as a feature selector that only retains variables with meaningful discretization bins. Biomedical datasets are high dimensional, there can be many noisy and redundant variables. Supervised discretization can help remove some of these variables from the model learning process. We discretized the dataset using efficient Bayesian discretization (EBD), a supervised discretization method, which has been shown to obtain better classification performance and stability but less robust when compared to the popular Fayyad-Irani supervised discretization method on several biomedical datasets[19]. We set the user-defined lambda parameter of EBD, to 0.5, as the recommended default value in the paper. During model learning, we split the data into 10 folds for cross-validation. For each train-test fold pair, supervised discretization bins were learned on the train dataset alone. The learned bins were applied to the test

Table 1 Clinical features of the 60 non-small cell lung cancer patients in the real-world lung cancer prognostic dataset

Attribute	Value	*n* (%)
Gender	Women	60 (100)
	Men	0 (0)
Tumor type	Adenocarcinoma	56 (93)
	Bronchioloalveolar carcinoma	3 (5)
	Squamous	1 (2)
	Others	0 (0)
Smoking history	Yes	0 (0)
	No	60 (100)

Statistics extracted from the paper by Lu *et al*[16].

dataset. So, during supervised discretization, we did not look at the test dataset.

Data background knowledge: We explored the medical literature for known prognostic markers that may assist in model learning with BRL$_p$. Before exploring, we first sought to understand more about the dataset, which turned out to have some interesting characteristics making it highly worthy of study. Of note, only tissue samples taken from non-smokers who were all women, who had contracted lung cancer were analyzed in this study. Table 1 summarizes some clinical features known about the 60 Taiwanese NSCLC patients studied in the dataset as described in the paper of the study[16].

We noted from the Table 1 that the subjects in the dataset were all women (60 out of 60 patients), contain mainly adenocarcinoma patients (56 out of 60 patients), and none of them had any smoking history (60 out of 60 patients). Additionally, we also knew that all the patients were from Taiwan. So, we explored the medical literature to find known prognostic markers for this sub-population. Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase is prognostic marker known to be frequently over-expressed in NSCLC[20]. EGFR encodes a transmembrane glycoprotein, a receptor for members of the epidermal growth factor family. A ligand binding to this receptor induces dimerization and tyrosine autophosphorylation, and leads to cell proliferation (referred from RefSeq, June 2016). In NSCLC patients, Shigematsu *et al*[21] observed that EGFR domain mutations are statistically significantly more frequent in women than men (42% *vs* 14%), in adenocarcinomas than other histologies (40% *vs* 3%), in non-smokers than smokers (51% *vs* 10%), and in East Asians than other ethnicities (30% *vs* 8%); all with a *P*-value of < 0.001. This description is very similar to the subjects in the dataset we are studying. Therefore, EGFR gene expression was potentially a good candidate to be incorporated as prior domain knowledge into model learning with BRL$_p$ on this dataset.

Methods compared: We again evaluated BRL$_p$ here. We set its of maximum conjuncts to 8. We evaluated the effect of the hyperparameter λ by assigning it values of $\lambda = \{0,1,2,4,6,8,10,20\}$. The value $\lambda = 0$ represents the baseline model of BRL with no structure priors. We included $\lambda = 20$, to study the scenario where the structure priors overwhelmingly dominates the likelihood score. Additionally, we compared these models with some state-of-the-art classifiers including three interpretable class of classifiers namely - C4.5[22], RIPPER[23], and PART[24]; and three complex and non-interpretable classifiers namely- Random Forests[25], naïve Bayes[26], and Support Vector Machines[27]. C4.5[22] is a popular decision tree learning algorithm, where each path of the decision tree can be interpreted as rules. RIPPER[23] (Repeated Incremental Pruning to Produce Error Reduction) is a propositional rule learning algorithm that uses a divide-and-conquer strategy during model training. PART[24] is a rule learning method that combines the approaches of both C4.5 and RIPPER by building partial decision trees, inferring rules from the trees, and using a divide-and-conquer strategy to build the rule model. Random Forest[25] is an ensemble learning method that learns a number of decision trees during training, and combines predictions from them during inference. The naive Bayes[26] classifier is a simple probabilistic classifier that learns a network with strong independence assumption between the variables, and uses the Bayes theorem for inference from the learned network. Support Vector Machines[27] is an algorithm that learns a hyperplane function to differentiate the classes in the problem space. We ran these classifiers from the Weka[28] workbench (version 3.8.1) using the default parameters for each classifier.

Evaluation metrics: We evaluated BRL$_p$ with two metrics: (1) Prior Frequency (PF); and (2) AUC. We evaluated the dataset over 5 runs of 10-fold cross-validation. For this real-world scenario, we used PF to measure the gain of the background knowledge into BRL$_p$. With the simulated dataset, we had evaluated using GED because we knew the true data-generating graph. In most real-world problems, we do not know the true model that generated the data and so, we cannot use GED. PF measures the fraction of models learned on each of the 50 folds (5 runs of 10-fold cross-validation) that incorporates the specified prior domain knowledge. In this experiment, we measured the fraction of the models that contained an edge between EGFR and *T* in the learned BRL$_p$ model.

RESULTS

In this section, we present the results from our experiments examining the effects of the λ hyperparameter of the structure prior, and consequentially the influence of the specified prior knowledge on model learning. We show our results using the simulated data first, and then from the real-world lung cancer prognostic dataset.

Simulation data analysis results

The results from the 5 runs of 10-fold cross-validation

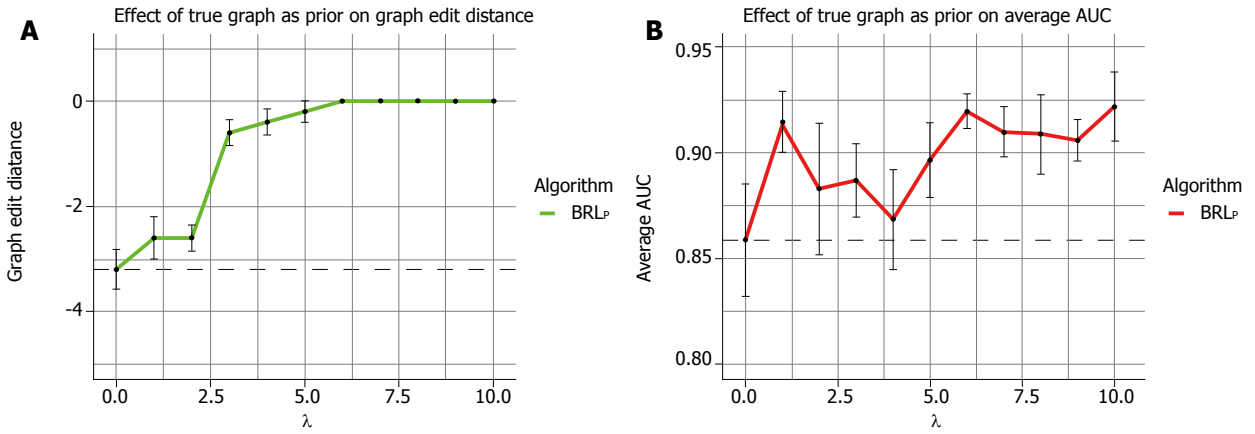

Figure 3 Evaluation metrics on Bayesian rule learning model learning with simulated data. A: Graph edit distance between BRLp and true data-generating model; B: Area under the receiver operator characteristic curve of the BRLp model. BRLp: Bayesian rule learning with informative priors; AUC: Area under the receiver operator characteristic curve.

1. IF (RV1000 = 0) THEN (T = 0)
Posterior Probability = 0.9944, TP = 44, FP = 0, Pos = 48, Neg = 52

2. IF (RV1000 = 1) THEN (T = 1)
Posterior Probability = 0.9248, TP = 52, FP = 4, Pos = 52, Neg = 48

Figure 4 Bayesian rule learning generated rule model with λ=10 (highest average area under the receiver operator characteristic curve) on the simulated dataset. Each rule has its posterior probability, the number of true positives (TP), false positives (FP), total number of examples that match the rules consequent target value (Pos), and total number that do not match the right hand side of the rule (Neg). The TP measures examples that correctly match the rules left and right hand sides, while FP measures examples that correctly match the rules condition or left-hand-side, but have a different consequent or right-hand-side.

are summarized in Figure 3. In Figure 3A, the various values of the hyperparameter λ is shown in the x-axis, while the y-axis shows the average GED. This average is obtained across the 10-folds of each run, and then averaged across the 5 runs. Each data-point in the graph is this average deviation from the true model as measured by the GED, and the error bars represent the standard error of mean. The dotted line shows the value of BRL$_P$ with λ = 0, which as we mentioned earlier is the same as BRL, where we use uninformative priors. We saw that even with λ = 1, the structure priors helped improve the GED thereby bringing the learned model closer to the data-generating model. We saw a sharp gain of GED from λ = 2 to 3. For λ ⩾ 6, BRL$_P$ returned the true data-generating model specified by the structure priors. This showed that BRL$_P$ effectively and correctly incorporates the specified domain knowledge. The degree of incorporation is controlled by λ.

Figure 3B displays the average AUC. The overall trend is a gain in AUC but the trend is noisy, especially with low λ values when the GED > 0. This region indicated models that picked up irrelevant variables, which were spurious and were associated with *T*, by chance. Their AUC fluctuated a lot because random associations were found. When λ ⩾ 6, the GED reached the perfect 0, we saw a rise in AUC. The noise reduced in this region of the graph. Random samplings from our simulation generated slightly different values of the parameters, which were reflected in the fluctuations here. So, from the AUC graph we saw a gradual gain in predictive performance with the incorporation of prior knowledge of the truth.

Figure 4 shows a BRL$_P$ rule model obtained when λ = 10, which achieved the largest average AUC from our experiments (AUC = 0.92). The particular run achieved an AUC of 0.96 on the 10-fold cross-validation and a GED of a perfect 0. The posterior probability was computed using Equation 6. TP and FP refers to the total true positives and false positives. Pos and Neg are the total positives and negative examples. Our simulation design only had one relevant variable, R_{1000}, and 999 irrelevant variables, $\{I_1 \ldots I_{1000}\}$. The rule model in Figure 4 correctly picked up only the relevant variable. We had designed the simulation such that if the relevant variable took the value 1, then *T* would be sampled with a Bernouli distribution with $p = 0.9$, this was reflected in Rule 2. So, BRL$_P$ accurately retrieved the true data-generating model assisted by informed structure priors.

Real-world lung cancer prognostic data analysis results

The results from the 5 runs of 10-fold cross-validation on the real-world lung cancer prognostic dataset are summarized in Figure 5. We specified the structure prior of an edge between EGFR and the outcome *Class* variable to be present. We altered the values of λ and observed its effect on the learned model. Figure 5A, shows the effect of the different values of λ on PF, the fraction of models that contained EGFR. From λ = 2 to 6,

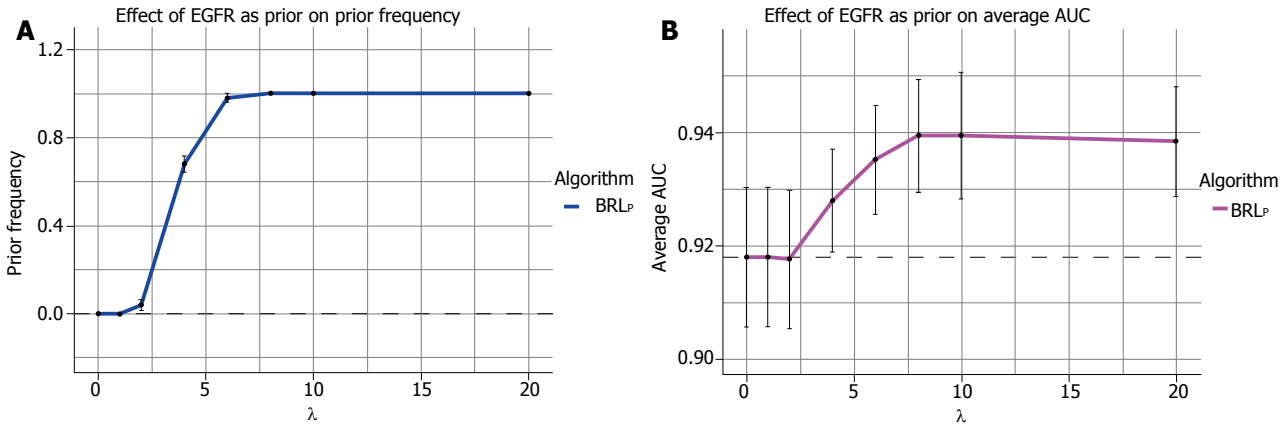

Figure 5 Evaluation metrics on Bayesian rule learning model learning with real-world lung cancer prognostic dataset. A: Prior frequency of the edge between epidermal growth factor receptor and T in BRLp model; B: Area under the receiver operator characteristic curve of the BRLp model. BRLp: Bayesian rule learning with informative priors; EGFR: Epidermal growth factor receptor; AUC: Area under the receiver operator characteristic curve.

we saw a steep gain in PF. For $\lambda \geqslant 8$, EGFR was present in every learned model. This again showed that BRL$_p$ effectively incorporated the specified prior knowledge and the λ hyperparameter allowed the user to determine the degree of incorporation of this knowledge by BRL$_p$.

Figure 5B, shows the gain of average AUC across 5 runs of 10-fold cross-validation. We observe a steady gain of AUC for $\lambda > 2$. For $\lambda \geqslant 8$, the AUC gain tapers off. The results show that the EGFR prior knowledge helped improve the AUC of BRL$_p$.

BRL$_p$ with $\lambda = 8$ generated the highest average AUC of 0.935. Figure 6 shows the rule model from one of the runs, which had achieved a cross-validation AUC of 0.967 and PF of 1. Rule 1 had the highest amount of evidence (38 true positives and no false positives) for the outcome Control (normal tissue). This rule had the EGFR value range from negative infinity to 10.8. In other words, EGFR was under-expressed in these 38 normal tissue instances. Rule 15 had the highest amount of evidence (15 true positives) for the outcome Case. This rule had EGFR value range from 10.8 to positive infinity. In other words, EGFR was over-expressed in these 15 tumor tissue instances. These rules also lent support to what we had found in the literature about EGFR being over-expressed in tumor cells. In addition to EGFR, which was incorporated from the structure prior, the model picked up 3 other variables during model learning from the dataset. They were ephrin A4 (EFNA4), killer cell lectin like receptor G2 (KLRG2), and C2 calcium dependent domain containing 6 (C2CD6).

Finally, we compared two BRL$_p$ models with state-of-the-art classifiers using average AUC achieved across 5 runs of 10-fold cross-validation. The two BRL$_p$ models were (1) with $\lambda = 0$, which represented the baseline BRL model with uninformative priors, and (2) with $\lambda = 8$ that incorporated EGFR into the structure prior, which achieved the highest average AUC of 0.935. The state-of-the-art classifiers compared were C4.5, RIPPER, PART, Random Forests, naïve Bayes, and Support Vector Machines. This comparison is shown in Figure 7.

The first two bars in Figure 7 are BRL$_p$ algorithms, BRL$_p$ with $\lambda = 0$ is indicated as BRL, and then BRL$_p$ with $\lambda = 8$. We saw a gain in performance crom incorporating EGFR as structure priors. The next three bars - C4.5, RIPPER, and PART are interpretable class of models, which are human readable. C4.5 is a decision tree learning algorithm. RIPPER and PART are rule learning algorithms. We noticed that these three algorithms performed worse than both BRL$_p$ algorithms in this dataset. The last three bars in Figure 7 are - Random Forest, naïve Bayes, and Support Vector Machines. These are examples of complex models that use all variables in the dataset to generate a classifier. It is not easy to explain the reasoning behind their predictions. But all three algorithms here outperformed BRL$_p$ on this dataset. This comparison shows the trade-off of predictive performance and interpretability. On this dataset, BRL$_p$ offered an interpretable model that outperformed other popular interpretable models but did not perform as well as the complex models.

DISCUSSION

An important practical consideration to note while specifying structure priors is to avoid specifying priors that introduce bias into the model search. Informative priors can be biased if they are inferred based on the predictions, of some predictive model, on the test dataset. For example, if we notice that our learned model predicts poorly on a subset of test instances, and we notice some independent variable(s) strongly associated with the target variable in that subset of test instances. Specifying, our newly found association from the predictions on the test dataset, into the structure priors to re-learn the model will return a biased model and must be avoided.

Mukherjee and Speed[8] show how the general form of the score in Equation 7 can be extended to

1.IF ((EFNA4 = -inf to 6.9) (KLRG2 = 6.4 to inf) (EGFR = -inf to 10.8) (C2CD6 = -inf to 3.9)) THEN (Class = Control)
Posterior Probability=0.9995, TP=38, FP=0, Pos=60, Neg=60

2.IF ((EFNA4 = 6.9 to 7.5) (KLRG2 = 6.4 to inf) (EGFR = -inf to 10.8) (C2CD6 = -inf to 3.9)) THEN (Class = Control)
Posterior Probability=0.9977, TP=9, FP=0, Pos=60, Neg=60

3.IF ((EFNA4 = -inf to 6.9) (KLRG2 = 6.4 to inf) (EGFR = 10.8 to inf) (C2CD6 = -inf to 3.9)) THEN (Class = Control)
Posterior Probability=0.9959, TP=5, FP=0, Pos=60, Neg=60

4.IF ((EFNA4 = -inf to 6.9) (KLRG2 = -inf to 6.4) (EGFR = -inf to 10.8) (C2CD6 = -inf to 3.9)) THEN (Class = Control)
Posterior Probability=0.9959, TP=5, FP=0, Pos=60, Neg=60

5.IF ((EFNA4 = 7.5 to inf) (KLRG2 = 6.4 to inf) (EGFR = 10.8 to inf) (C2CD6 = 3.9 to inf)) THEN (Class = Control)
Posterior Probability=0.9898, TP=2, FP=0, Pos=60, Neg=60

6.IF ((EFNA4 = 6.9 to 7.5) (KLRG2 = 6.4 to inf) (EGFR = 10.8 to inf) (C2CD6 = -inf to 3.9)) THEN (Class = Control)
Posterior Probability=0.98, TP=1, FP=0, Pos=60, Neg=60

Rules 7 through 14 match 0 instances and so are removed from display.

15.IF ((EFNA4 = 7.5 to inf) (KLRG2 = -inf to 6.4) (EGFR = 10.8 to inf) (C2CD6 = -inf to 3.9)) THEN (Class = Case)
Posterior Probability=0.9986, TP=15, FP=0, Pos=60, Neg=60

16.IF ((EFNA4 = 7.5 to inf) (KLRG2 = -inf to 6.4) (EGFR = -inf to 10.8) (C2CD6 = -inf to 3.9)) THEN (Class = Case)
Posterior Probability=0.9985, TP=14, FP=0, Pos=60, Neg=60

17.IF ((EFNA4 = 7.5 to inf) (KLRG2 = 6.4 to inf) (EGFR = 10.8 to inf) (C2CD6 = -inf to 3.9)) THEN (Class = Case)
Posterior Probability=0.9974, TP=8, FP=0, Pos=60, Neg=60

18.IF ((EFNA4 = 7.5 to inf) (KLRG2 = -inf to 6.4) (EGFR = 10.8 to inf) (C2CD6 = 3.9 to inf)) THEN (Class = Case)
Posterior Probability=0.997, TP=7, FP=0, Pos=60, Neg=60

19.IF ((EFNA4 = 7.5 to inf) (KLRG2 = -inf to 6.4) (EGFR = -inf to 10.8) (C2CD6 = 3.9 to inf)) THEN (Class = Case)
Posterior Probability=0.9959, TP=5, FP=0, Pos=60, Neg=60

20.IF ((EFNA4 = 7.5 to inf) (KLRG2 = 6.4 to inf) (EGFR = -inf to 10.8) (C2CD6 = -inf to 3.9)) THEN (Class = Case)
Posterior Probability=0.9948, TP=4, FP=0, Pos=60, Neg=60

21.IF ((EFNA4 = 6.9 to 7.5) (KLRG2 = -inf to 6.4) (EGFR = 10.8 to inf) (C2CD6 = -inf to 3.9)) THEN (Class = Case)
Posterior Probability=0.9932, TP=3, FP=0, Pos=60, Neg=60

22.IF ((EFNA4 = 7.5 to inf) (KLRG2 = 6.4 to inf) (EGFR = -inf to 10.8) (C2CD6 = 3.9 to inf)) THEN (Class = Case)
Posterior Probability=0.9898, TP=2, FP=0, Pos=60, Neg=60

23.IF ((EFNA4 = 6.9 to 7.5) (KLRG2 = -inf to 6.4) (EGFR = -inf to 10.8) (C2CD6 = 3.9 to inf)) THEN (Class = Case)
Posterior Probability=0.98, TP=1, FP=0, Pos=60, Neg=60

24.IF ((EFNA4 = 6.9 to 7.5) (KLRG2 = -inf to 6.4) (EGFR = 10.8 to inf) (C2CD6 = 3.9 to inf)) THEN (Class = Case)
Posterior Probability=0.98, TP=1, FP=0, Pos=60, Neg=60

Figure 6 Bayesian rule learning generated rule model with λ=8 (highest average area under the receiver operator characteristics curve) on the real-world lung cancer prognostic dataset. TP: True positives; FP: False positives; Pos: Total number of examples that match the rules consequent target value; Neg: Total number that do not match the right hand side of the rule; EGFR: Epidermal growth factor receptor.

incorporate other kinds of prior knowledge including rewarding network sparsity, where structure priors can be used as a regularization term. In the introduction section, we had discussed other sources of prior knowledge than literature, including - input from a domain expert (*e.g.*, A physician), domain ontology (*e.g.*, Gene Ontology), and models learned from other related datasets. In the future, we will explore the incorporation of knowledge from these other sources. In novel biomarker discovery, we could place all of our known knowledge into the negative edge-set in Equation 8. Models learned from such a structure prior would be penalized for learning already known biomarkers and would encourage discovery of novel biomarkers. We used an instantiation of the general form of the score, in Equation 7, where the relative weights, w_i, of each of i^{th} network are set to 1. It would be interesting to explore different relative weights for different network features and see its impact on model learning. In this paper, we performed a grid search over the hyperparameter λ. We would like to explore if we can come up with better ways to optimize the value of this hyperparameter.

In this paper, we implemented BRL_P, a method

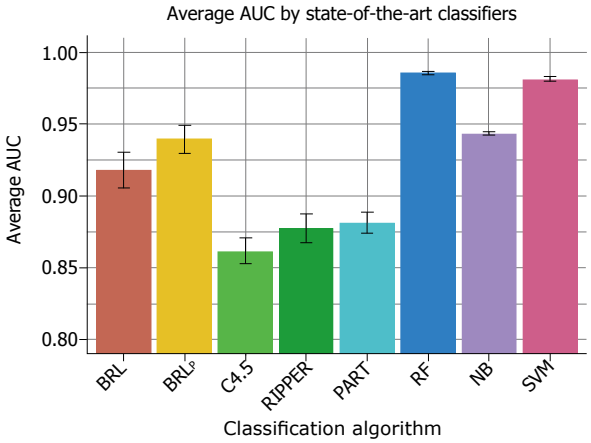

Figure 7 Comparison of area under the receiver operator characteristics curve achieved by Bayesian rule learning with state-of-the-art classifiers. AUC: Area under the receiver operator characteristic curve.

that extended BRL to allow it to integrate prior domain knowledge using structure priors into the model learning process. We demonstrated the ability of BRLp to incorporate this knowledge on simulated data and a real-world lung cancer prognostic dataset. We observed that the λ hyperparameter allowed us to control the degree of incorporation of prior knowledge. This parameter can be helpful if we were uncertain about our specified prior knowledge. We also observed that relevant prior knowledge could sometimes help improve the predictive performance of BRLp. Methods developed in this paper, the simulation data experiment code, and the R script for data extraction and processing of the prognostic dataset, are all made publicly available in an online repository (https://github.com/jeya-pitt/brl-structure-priors). We envision that BRLp will be very beneficial in data mining tasks across domains where some prior domain knowledge is available.

ARTICLE HIGHLIGHTS

Research background

Biomedicine is increasingly a data-driven science, owing largely to the explosion in data, especially from the development of high-throughput technologies. Such datasets often suffer from the problem of high-dimensionality, where a very large number of candidate variables can explain the outcome variable of interest but have few instances to support any model hypothesis. In many applications, in addition to the data itself, some domain knowledge is available that may assist in the data mining process to help learn more meaningful models. It is important to develop data mining tools to leverage this available domain knowledge. However, currently, there is a dearth of data mining methods that can incorporate this available domain knowledge.

Research motivation

Developing data mining methods that can incorporate domain knowledge will help learn more meaningful models and will benefit many domains, especially the ones that suffer from data scarcity but have some domain knowledge that can assist with the data mining process (for example - biomedicine).

Research objectives

In this work, our objective was to extend a rule learning algorithm, called Bayesian rule learning (BRL), to make it capable of incorporating prior domain knowledge. BRL is a good candidate because it has been shown to be successful in application to high-dimensional biomedical data analysis tasks. We implemented such a tool, called BRLp that has tunable priors, which means the user can control the degree of incorporation of their specified knowledge. BRLp is a novel data mining tool that allows the user to specify their domain knowledge (including uncertain domain knowledge) and incorporates it into the model search process.

Research methods

BRL searches over a space of Bayesian belief network models (BNs) to find the optimal network and infers a rule set from that model. We implemented a way for the BN to incorporate informative priors, a distribution encoding the relative importance of each model prior to seeing the training data. This allowed BRL to incorporate user-specified domain knowledge into the data mining process called BRLp. BRLp has a hyperparameter λ that allows the user to adjust the degree of incorporation of their specified prior knowledge.

We evaluated BRLp by comparing it to BRL (without informative priors) and other state-of-the-art classifiers on a simple simulated dataset, and a real-world lung cancer prognostic dataset. We measured the degree of acceptance of the specified prior knowledge with respect to the hyperparameter λ in BRLp. We also observed the changes in predictive power using AUC.

Research results

We observed, in both the experiments with simulated data and the real-world lung cancer prognostic data that with increasing values of λ the degree of incorporation of the specified prior knowledge also increased. We also observed that specifying prior knowledge relevant to the problem dataset could sometimes help find models with better predictive performance. When BRLp is compared to the state-of-the-art classifiers, we observed that it performed better than other interpretable models but the more complex and non-interpretable models achieved better predictive performance than BRLp.

Research conclusions

BRLp allows the user to incorporate their specified domain knowledge into the data mining task and allows them to control the degree of incorporation with a hyperparameter. This is a novel rule learning algorithm that we have made available to the general public via GitHub. We anticipate its use in many applications especially the ones suffering from data scarcity but have additional domain knowledge available that may assist in the data mining task.

Research perspectives

In this paper, we explored specifications of simple domain knowledge. We need to further explore the incorporation of more complex forms of knowledge. In this paper, we incorporate domain knowledge from literature. We also want to explore domain knowledge available in other sources. These future directions may motivate further developments to BRLp.

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P- Reviewer: Gadbail AR, To KKW, Yao DF **S- Editor**: Ma YJ
L- Editor: A **E- Editor**: Wu YXJ

World Journal of Clinical Oncology

Submit a Manuscript: http://www.f6publishing.com

World J Clin Oncol 2018 September 14; 9(5): 110-118

DOI: 10.5306/wjco.v9.i5.110

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

FOLFIRI3-aflibercept in previously treated patients with metastatic colorectal cancer

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Author contributions: Carola C, Barlet J and Chibaudel B completed the data collection and prepared the manuscript; Carola C, de Gramont A and Chibaudel B analyzed the data; all authors had read and approved manuscript.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Committee in Cancer Research (IRCCR) of Franco-British Hospital (FBI). Personal and filiation data including identity of every patient was protected with an added code in the Excel table. This is a retrospective case series that did not have any activity or contact with the patients.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment.

Conflict-of-interest statement: Dr. Chibaudel has nothing to disclose.

STROBE statement: The authors have read the STROBE Statement and the manuscript was prepared and revised according to the STROBE Statement.

Manuscript source: Invited manuscript

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Received: March 30, 2018
Peer-review started: March 30, 2018
First decision: May 2, 2018
Revised: June 11, 2018
Accepted: June 28, 2018
Article in press: June 28, 2018
Published online: September 14, 2018

Abstract

AIM

To evaluate the efficacy and safety of the modified FOLFIRI3-aflibercept as second-line therapy in patients with metastatic colorectal cancer.

METHODS

This is a retrospective multicenter cohort, evaluating the efficacy and safety of the association of aflibercept with FOLFIRI3 (day 1: aflibercept 4 mg/kg, folinic acid 400 mg/m^2, irinotecan 90 mg/m^2, 5-fluorouracil infusion 2400 mg/m^2 per 46 h; day 3: irinotecan 90 mg/m^2) in patients with previously treated metastatic colorectal cancer. The primary endpoint was overall response rate (ORR). Secondary endpoints were disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety.

RESULTS

Among 74 patients treated in four French centers, nine were excluded due to prior use of aflibercept (n = 3), more than one prior treatment line in irinotecan-naïve patients (n = 3), and inadequate liver function (n = 3). In the "irinotecan-naïve" patients (n = 30), ORR was 43.3% and DCR was 76.7%. Median PFS and OS were 11.3 mo (95%CI: 6.1-29.0) and 17.0 mo (95%CI: 13.0-17.3), respectively. The most common (> 5%) grade 3-4 adverse events were diarrhea (37.9%), neutropenia (14.3%), stomatitis and anemia (10.4%), and hypertension (6.7%). In the "pre-exposed irinotecan" patients (n = 35), 20 (57.1%) received $\geqslant$ 2 prior lines of treatment. ORR was 34.3% and DCR was 60.0%. Median PFS and OS were 5.7 mo (95%CI: 3.9-10.4) and 14.3 mo (95%CI: 12.8-19.5), respectively.

CONCLUSION

Minimally modified FOLFIRI has improvement dramatically the FOLFIRI3-aflibercept efficacy, whatever prior use of irinotecan. A prospective randomized trial is warranted to compare FOLFIRI-aflibercept to FOLFIRI3-aflibercept.

Key words: Chemotherapy; Irinotecan; Aflibercept; Second-line; Colorectal cancer

Core tip: Results obtained in this retrospective study show that minimally modified FOLFIRI has improvement dramatically the efficacy of the FOLFIRI3-aflibercept combination with high response rates and survivals in patients with previously treated metastatic colorectal cancer, whatever prior use of irinotecan. A prospective randomized trial is planned to compare FOLFIRI-aflibercept to FOLFIRI3-aflibercept.

Carola C, Ghiringhelli F, Kim S, André T, Barlet J, Bengrine-Lefevre L, Marijon H, Garcia-Larnicol ML, Borg C, Dainese L, Steuer N, Richa H, Benetkiewicz M, Larsen AK, de Gramont A, Chibaudel B. FOLFIRI3-aflibercept in previously treated patients with metastatic colorectal cancer. *World J Clin Oncol* 2018; 9(5): 110-118 Available from: URL: http://www.wjgnet.com/2218-4333/full/v9/i5/110.htm DOI: http://dx.doi.org/10.5306/wjco.v9.i5.110

INTRODUCTION

Standard second-line therapy in patients with previously treated metastatic colorectal cancer (mCRC) is doublet fluoropyrimidine-based chemotherapy with either irinotecan (FOLFIRI) or oxaliplatin (FOLFOX), depending on the regimen used in first-line, in association with antiangiogenic agents (*e.g.*, bevacizumab, aflibercept, ramucirumab) or anti-EGFR agents in absence of *RAS* tumor gene mutation (*e.g.*, cetuximab, panitumumab)[1-8].

The standard FOLFIRI regimen was optimized by splitting the dose of irinotecan on day 1 [half dose before 5-fluorouracil (5-FU)] and day 3 (half dose after 5-FU) in the so-called FOLFIRI3 regimen[9]. Drugs and doses are similar to FOLFIRI, except for suppression of the 5-FU bolus. The response rate was higher than that reported for FOLFIRI[9,10]. Based on these results, FOLFIRI3 became the second-line regimen of choice in some centers. Adding bevacizumab to FOLFIRI3 has shown promising efficacy results in two prior retrospective trials [response rate 22% and 35%, median progression-free survival (PFS) 7.0 and 6.2 mo, median overall survival (OS) 13.0 and 10.8 mo, respectively][11,12]. The addition of aflibercept to FOLFIRI in patients with pretreated mCRC increased response rate from 11% to 20% and improved median PFS from 4.7 to 6.9 mo [hazard ratio (HR) = 0.76] and median OS from 12.1 to 13.5 mo (HR = 0.82)[4]. Aflibercept was approved by the Food and Drug Administration on August 3, 2012 and by the European Medicines Agency on February 1, 2013 in combination with FOLFIRI for the treatment of patients with mCRC resistant to an oxaliplatin-containing regimen[13]. Based on the VELOUR study results and non-randomized FOLFIRI3 studies, we retrospectively analyzed the safety and efficacy of the FOLFIRI3-aflibercept combination as second or later-line therapy in patients with mCRC.

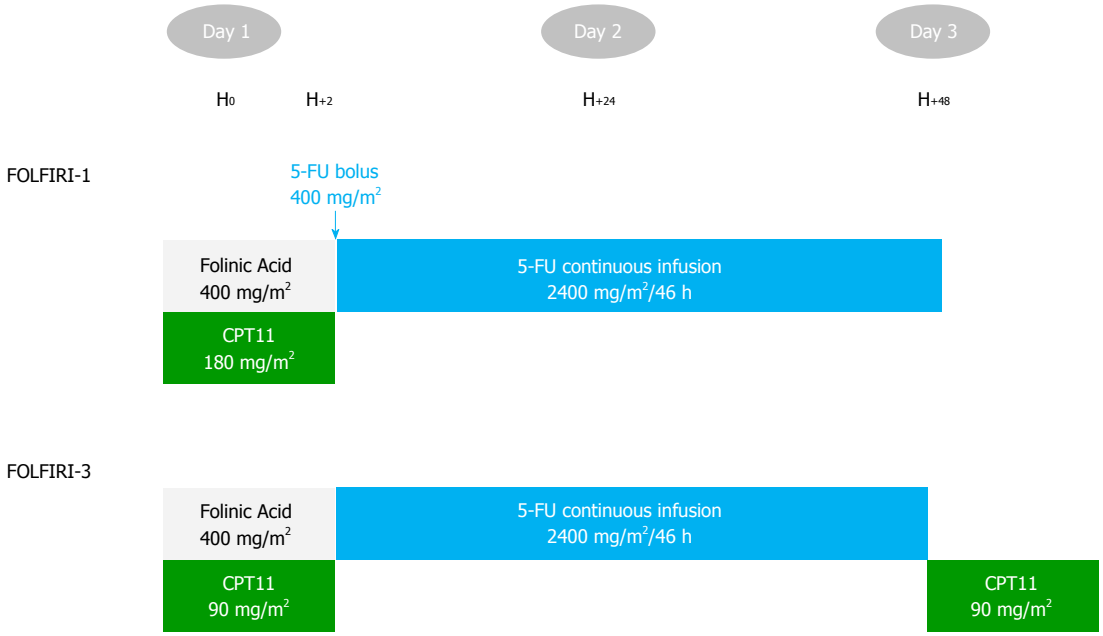

Figure 1 Comparison of the FOLFIRI-1and FOLFIRI-3 schedules.

MATERIALS AND METHODS

This study was a retrospective, multicenter cohort, conducted in four French institutions (Centre Georges François Leclerc, Franco-British Hospital, University Hospital Besançon, and Saint-Antoine University Hospital) from September 2014 to December 2016.The main objective of this study was to evaluate the efficacy and safety profile of the aflibercept-FOLFIRI3 combination.

Population

All patients with previously treated mCRC and with FOLFIRI3-aflibercept administered from September 2014 to December 2016 were included. During the inclusion period, the decision to give FOLFIRI3-aflibercept to each patient or another treatment regimen was at physician's discretion. Prior use of bevacizumab was allowed, but prior exposure to aflibercept was not permitted. Patients were divided into two subgroups depending on the prior use of irinotecan and the number of previous treatment lines for metastatic disease: (1) "irinotecan-naive" population including patients with no more than one prior line of treatment for metastatic disease; and (2) the "irinotecan pre-exposed" population including patients for whom the number of prior treatment lines for metastatic disease was not restricted.

Treatment administration

Treatment cycles were given intravenously every 14 d, as follows: Aflibercept 4 mg/kg over 1-h infusion (day 1), folinic acid 400 mg/m² over 2-h infusion (day 1), irinotecan 90 mg/m² over 60-90 min infusion (day 1), followed by continuous 5-FU 2400 mg/m² as a 46-h infusion (days 1 to 3), then irinotecan 90 mg/m² over 60-90 min infusion (day 3; Figure 1). Treatment information (date of treatment, doses) was collected using CHIMIO® 5.4 (Computer Engineering, Paris, France) or BPC (GCS Emosist, Région Franche-Comté, France) softwares.

Endpoints

Treatment efficacy was evaluated with tumor response, PFS, and OS. The objective response rate (ORR) was defined as the proportion of patients having either complete response (CR) or partial response (PR) according to RECIST version 1.1. The best ORR was defined as the best response recorded from the start of treatment until progressive disease (PD). Disease control rate (DCR) was the sum of ORR and stable disease (SD). PFS was defined as the time from the date of starting treatment to the date of progression or death (from any cause). OS was defined as the time from the date of starting treatment to the date of patient death (from any cause) or to the last date the patient was known to be alive. Toxicity was evaluated according to the United States National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03.

Statistical analysis

Follow-up and survival were estimated using the reverse Kaplan-Meier method and the Kaplan-Meier method, respectively, and were described using median with 95% confidence interval (CI). Qualitative variables were described using percent and means and continuous variables using medians (minimum-maximum). The cut-off date for statistical analysis was June 15, 2017. The final analysis was performed on the irinotecan-naïve and irinotecan pre-exposed

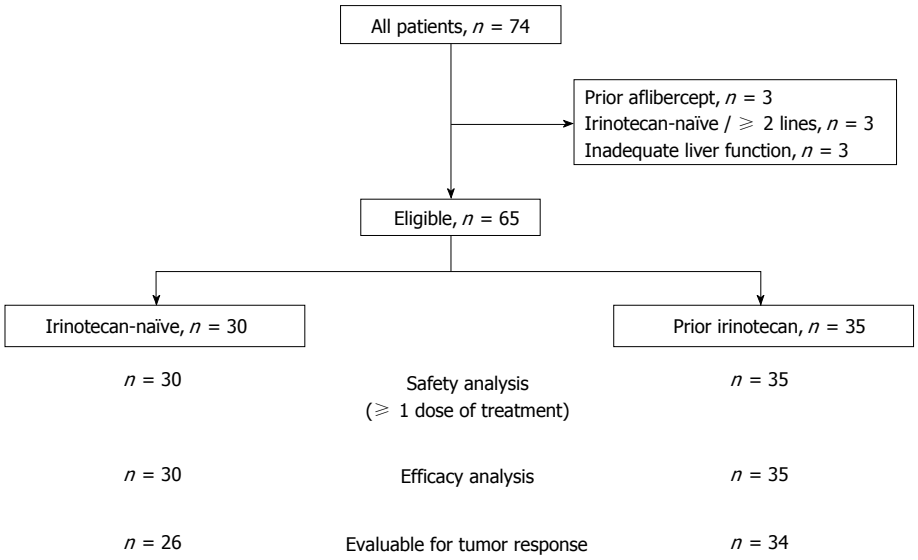

Figure 2 Flow diagram.

populations.

RESULTS

A total of 74 patients were treated (Figure 2). Nine patients were excluded from the analysis due to: Prior use of aflibercept (n = 3), more than one prior line of treatment in irinotecan-naïve patients (n = 3), or inadequate liver function (pretreatment alkaline phosphatase level > 7 × upper normal limit, n = 3). Thirty patients did not receive prior irinotecan (the irinotecan-naïve population) and 35 were previously exposed to irinotecan (the pre-exposed population).

Overall, 25 (38.5%) patients had an Eastern Cooperative Oncology Group performance status 0. The mean age was 63.1 years (range: 31.9-82.1); 23 (35.4%) had a single metastatic site, 49 (75.4%) had *RAS* mutated tumors, and two (3.1%) had *BRAF* mutated tumors. Prior use of bevacizumab and anti-EGFR were reported in 47 (72.3%) and 6 (9.2%) patients, respectively.

In the irinotecan-naïve population, five patients did not receive first-line therapy for metastatic disease (n = 4, early relapse after FOLFOX adjuvant therapy or n = 1, radiochemotherapy). In irinotecan pre-exposed population, 20 (57.1%) patients received more than two prior lines of treatment. Various irinotecan regimens (FOLFIRINOX, n = 21; FOLFIRI, n = 10; FOLFIRI3, n = 4) were previously given. The portion of patients with increased level of lactate dehydrogenase in the irinotecan pre-exposed population was higher than in that with the irinotecan-naïve patients (56.0% *vs* 17.6%; P = 0.027; Table 1).

Treatment exposure

In the irinotecan-naïve population, chemotherapy drugs (irinotecan and 5-FU) were given at standard dose in 12 (40.0%) patients. A lower dose of irinotecan and 5-FU were given in 15 (50.0%) and 4 (13.3%) patients, respectively. The median number of cycles was 8 (range: 1-19), and the median treatment duration was 3.7 mo (95%CI: 2.4-5.7). Dose reductions during treatment were performed in 7 (23.3%), 13 (43.3%), and 6 (20.0%) patients for aflibercept, irinotecan, and 5-FU, respectively. Granulocyte colony-stimulating factor (G-CSF) was given as primary prophylaxis in 14 (46.7%) patients and as secondary prevention in 3 (10.0%) patients. Erythropoietin was used in 5 (17.9%) patients. At the time of analysis, the treatment was still ongoing in 2 patients. The main reasons for stopping therapy were the occurrence of a limiting adverse event in 14 (46.7%) patients (diarrhea, n = 8; bleeding, n = 1; bowel perforation, n = 1; asthenia, n = 1; other, n = 3) or progression in 11 (36.7%) patients.

In the irinotecan pre-exposed population, chemotherapy drugs (irinotecan and 5-FU) were given at standard dose in 22 (62.9%) patients. A lower dose of irinotecan and 5-FU were given in 10 (28.6%), and 6 (17.1%) patients, respectively. The median number of cycles was 6 (range: 1-20), and the median treatment duration was 3.5 mo (95%CI: 2.1-5.6). Dose reductions during treatment were performed in 5 (14.3%), 9 (25.7%), and 7 (20.0%) patients for aflibercept, irinotecan, and 5-FU, respectively. G-CSF was given as primary prophylaxis in 13 (37.1%) patients and as secondary prevention in 2 (5.7%) patients. Erythropoietin was used in 3 (8.6%) patients. At the time of analysis, the treatment was still ongoing in 5 patients. The main reasons for stopping therapy were disease progression in 22 (62.9%) patients and the occurrence of limiting adverse events in 3 (8.6%) patients (diarrhea, n = 2; skin reactions, n = 1).

Response rate

In the irinotecan-naïve population, 4 (13.3%) patients

Table 1 Patient characteristics *n* (%)

Characteristics	Irinotecan-naive cohort	Irinotecan-pre-exposed cohort	*P*-value
Age (yr)			0.793
< 70	21 (70.0)	23 (65.7)	
≥ 70	9 (30.0)	12 (34.3)	
Sex			0.623
Male	16 (53.3)	21 (60.0)	
Female	14 (46.7)	14 (40.0)	
ECOG PS			0.227
0	14 (46.7)	11 (31.4)	
1	13 (43.3)	15 (42.9)	
2	3 (10.0)	9 (25.7)	
Time to metastasis			0.314
Metachronous	9 (30.0)	15 (42.9)	
Synchronous	21 (70.0)	20 (57.1)	
No. of metastatic sites			0.118
1	14 (46.7)	9 (25.7)	
> 1	16 (53.3)	26 (74.3)	
No. of prior lines for metastatic disease			-
0	5 (16.7)	0 (0.0)	
1	25 (83.3)	15 (42.9)	
> 1	-	20 (57.1)	
Prior drug exposure			0.699
Oxaliplatin	29 (96.7)	35 (100.0)	
Bevacizumab	18 (60.0)	29 (82.9)	
Anti-EGFR	2 (6.7)	4 (11.4)	

ECOG PS: Eastern Cooperative Oncology Group performance status.

Table 2 Summary of the efficacy results

	Irinotecan-naïve, *n* = 30			Prior irinotecan, *n* = 35		
Response rate						
	n	% (ITT)	% (evaluable)	*n*	% (ITT)	% (evaluable)
CR	0	0	0	1	2.8	2.9
PR	13	43.3	50	11	31.4	32.4
SD	10	33.3	38.5	9	25.7	26.5
PD	3	10	11.5	13	37.1	38.2
NE	4	13.3	-	1	2.8	-
ORR	13	43.3	50	12	34.3	35.3
DCR	23	76.7	88.5	21	60	61.8
Survivals						
	median, mo	95%CI		median, mo	95%CI	
PFS	11.3	6.1-29.0		5.7	3.9-10.4	
OS	17.0	13.0-17.3		14.3	12.8-19.5	

RR: Response rate; ITT: Intention-to-treat; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; NE: Not evaluable; ORR: Objective response rate; DCR: Disease control rate; PFS: Progression-free survival; OS: Overall survival.

were not evaluated for tumor response due to an early stop for limiting toxicity. ORR was reported in 13 patients [43.3%, intention-to-treat (ITT); 50.0%, evaluable patients] without CR, and DCR was reported in 23 patients (76.7%, ITT; 88.5%, evaluable patients). Three (10.0%) patients had PD at the first tumor evaluation (Table 2).

In the irinotecan pre-exposed population, one patient was not evaluable for tumor response (switch to intra-arterial chemotherapy after 2 treatment cycles). ORR was reported in 12 patients (34.3%, ITT; 35.3%, evaluable patients) including one CR, and DCR was reported in 21 patients (60.0%, ITT; 61.8%, evaluable population). Thirteen (37.1%) patients had PD at the first tumor evaluation (Table 2). Among seven patients refractory to irinotecan, 1 (2.9%) had PR with FOLFIRI3-aflibercept, 2 (5.7%) had stable disease, 3 (8.6%) had PD, and 1 (2.6%) was not evaluable (Table 3).

Survival

The median follow-up was 13.6 mo (95%CI: 9.6-17.7) in the irinotecan-naïve population and 14.2 mo (95%CI: 11.0-21.5) in the pre-exposed population (*P*

Table 3 Contingency table of tumor response with FOLFIRI3-aflibercept according to prior tumor response with irinotecan [*n* = 35, *n* (%)]

		FOLFIRI3-aflibercept				
		CR/PR	SD	PD	NE	All
Prior irinotecan-based regimen	**CR/PR**	5	3	7	0	15 (42.8)
	SD	4	4	2	0	10 (28.6)
	PD	1	2	3	1	7 (20.0)
	NE	2	0	1	0	3 (8.6)
	All	12 (34.3)	9 (25.7)	13 (37.1)	1 (2.8)	**35**

CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; NE: Not evaluable.

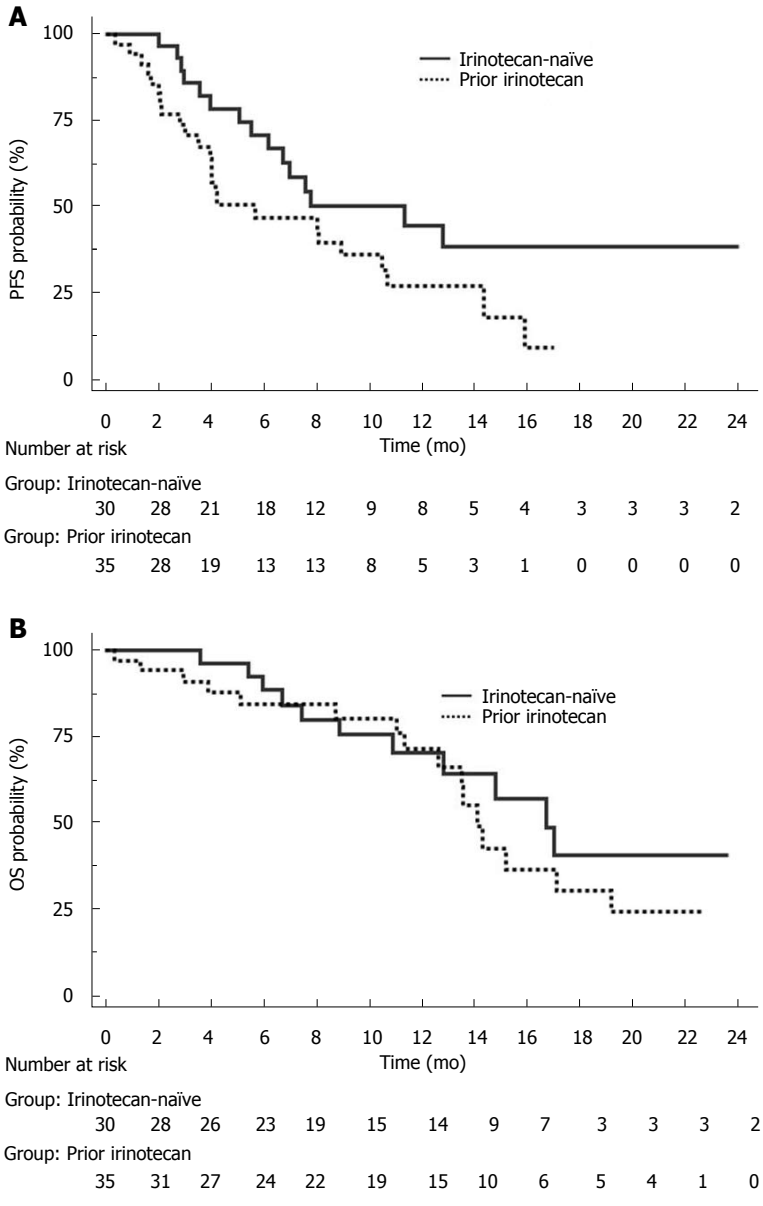

Figure 3 Progression-free survival and overall survival according to prior exposure to irinotecan (*n* = 65). A: Progression-free survival; B: Overall survival.

= 0.692). In the irinotecan-naïve population, median PFS was 11.3 mo (95%CI: 6.1-29.0) and median OS was 17.0 mo (95%CI: 13.0-17.3; Figure 3A). A lower starting dose of irinotecan (< 90 mg/m²) did not impact PFS (P = 0.518) and OS (P = 0.311), but decreased the incidence of severe neutropenia (0.0% *vs* 30.8%, respectively, P = 0.041). In the irinotecan pre-exposed population, median PFS was 5.7 mo (95%CI: 3.9-10.4) and median OS was 14.3 mo (95%CI: 12.8 -19.5; Figure 3B).

Table 4 Selected (≥ 5%) grade 3-4 adverse events (NCI CTCAE version 4.0) *n* (%)

SOC	PT	Irinotecan-naïve	Prior irinotecan	All
Any		17 (56.7)	15 (42.9)	32 (49.2)
Blood	Neutropenia	4 (13.3)	1 (2.9)	5 (7.7)
	Anemia	3 (10.0)	0 (0.0)	3 (4.6)
	Thrombocytopenia	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal	Nausea	2 (6.7)	0 (0.0)	2 (3.1)
	Vomiting	1 (3.3)	0 (0.0)	1 (1.5)
	Mucositis	3 (10.0)	3 (8.6)	6 (9.2)
	Diarrhea	11 (36.7)	9 (25.7)	20 (30.8)
Vascular	Hypertension	2 (6.7)	4 (11.4)	6 (9.2)

SOC: System Organ Class; PT: Preferred term.

Safety

In the irinotecan-naïve cohort, 17 (56.7%) patients experienced grade ≥ 3 toxicity (Table 4). The most common (≥ 5%) grade 3-4 adverse events were diarrhea (*n* = 11, 36.7%), neutropenia (*n* = 4, 13.3%), anemia and mucositis (*n* = 3, 10.0%), and nausea and hypertension (*n* = 2, 6.7%). Any grade hemorrhage was reported in 4 (13.8%) patients, gastrointestinal perforation in 1 (3.3%) patient, and arterial thromboembolic event in 1 (3.3%) patient.

In the irinotecan pre-exposed cohort, 15 (42.9%) patients experienced grade ≥ 3 toxicity (Table 4). The most common (≥ 5%) grade 3-4 adverse events were diarrhea (*n* = 9, 25.7%), hypertension (*n* = 4, 11.4%), and mucositis (*n* = 3, 8.6%).

Salvage surgery

Salvage surgery for metastatic disease was performed in 7 (10.0%) patients (*n* = 4, liver; *n* = 1, lung; *n* = 1, liver and lung; *n* = 1, peritoneum). A complete (R0) resection and liver pathological complete response were observed in all and one patient, respectively.

DISCUSSION

To our knowledge, this is the first report evaluating the FOLFIRI3-aflibercept combination in patients with previously treated mCRC. The response rate, which is a strong indicator of treatment efficacy, was unusually high not only in irinotecan-naïve patients (43%, ITT; 50%, evaluable), but also in irinotecan pre-exposed patients (34%, ITT, 35%, evaluable).

The median 11.3 mo PFS and median 17.0 mo OS in the irinotecan-naïve population receiving FOLFIRI3-aflibercept as second-line therapy compared favorably to the FOLFIRI-aflibercept combination in the pivotal phase Ⅲ VELOUR study (response rate 19.8%, median 7.2 mo PFS, median 13.2 mo OS) and the FOLFIRI3 regimen without targeted agent (response rate 17%-23%, median 4-7 mo PFS, median 9-12 mo OS)[4,9,10,14].

In the irinotecan pre-exposed population, patients received FOLFIRI3-aflibercept as salvage therapy. Yet, median PFS and OS were 5.7 mo and 14.3 mo, respectively, and were comparable to figures observed in second-line trials[8,15].

A high portion (27%) of patients had to stop the FOLFIRI3-aflibercept combination because of limiting toxicity, mainly diarrhea. Its frequency (38%) was twice as common as in the VELOUR study (19%), but in the same range as in previous studies using FOLFIRI3. It has been demonstrated that severe diarrhea induced by aflibercept is due to microscopic colitis, which can be managed successfully using oral budesonide and/or mesalamine treatment[16,17]. Placental growth factor (PlGF) could play a role in the occurrence of diarrhea. The absence of PlGF blocks dextransodium sulfate-induced colonic mucosal angiogenesis and increases mucosal hypoxia[18,19]. Knockout of PlGF aggravates disease course in acute colitis[20]. Neutropenia and stomatitis were at a lower incidence than in the FOLFIRI-aflibercept arm of the pivotal VELOUR study (13.3% *vs* 36.7%, neutropenia; 10.0% *vs* 13.8%, stomatitis), which can be explained by deletion of the 5-FU bolus in the FOLFIRI3 regimen and use of G-CSF in 49% of patients. In the irinotecan-naïve population, a lower starting dose of irinotecan (< 90 mg/m²) did not impact treatment efficacy, but decreased the incidence of severe neutropenia (0.0% *vs* 30.8%, *P* = 0.041).

The conversion to surgery of metastasis in second-line is another key finding that could modify the strategy in patients suitable for salvage surgery in case of response (sequential doublets versus triplets). The main limitation of this study is the retrospective design with a low number of patients. In conclusion, the combination of aflibercept and FOLFIRI3 in our study shows the encouraging efficacy results with high response rates and longer survivals in patients with previously treated mCRC, whatever the prior exposition to irinotecan. A randomized trial is warranted to compare FOLFIRI-aflibercept to FOLFIRI3-aflibercept.

ARTICLE HIGHLIGHTS

Research background

FOLFIRI3 is the second-line regimen of choice in patients with previously treated mCRC in some centers. Adding bevacizumab to FOLFIRI3 has shown

promising efficacy results in two prior retrospective trials. The addition of aflibercept to FOLFIRI in patients with pretreated mCRC increased response rate from 11% to 20% and improved median PFS from 4.7 to 6.9 mo.

Research motivation

The phase III VELOUR and non-randomized FOLFIRI3 studies results provide a backbone for our study.

Research objectives

The main objective of the study is to evaluate the safety and efficacy of the FOLFIRI3-aflibercept combination as second or later-line therapy in patients with mCRC.

Research methods

Patients with previously treated mCRC were given the aflibercept-FOLFIRI3 combination and were divided into "irinotecan-naive" population including patients with no more than one prior line of treatment for metastatic disease, and the "irinotecan pre-exposed" population including patients for whom the number of prior treatment lines for metastatic disease was not restricted. The primary endpoint was overall response rate (ORR). Secondary endpoints were disease control rate, progression-free survival (PFS), overall survival (OS), and safety. Toxicity was evaluated according to the United States National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03.

Research results

Minimally modified FOLFIRI has improvement dramatically the efficacy of the FOLFIRI3-aflibercept combination with high response rates (43% in irinotecan-naïve patients and 34% in irinotecan pre-exposed patients) and survivals (median PFS: 11.3 mo, OS: 17.0 mo and PFS: 5.7 mo and OS: 14.3 mo, respectively) in patients with previously treated mCRC, whatever prior use of irinotecan.

Research conclusions

The combination of aflibercept and FOLFIRI3 shows encouraging efficacy results in patients with previously treated mCRC.

Research perspectives

A prospective randomized trial is planned to compare FOLFIRI-aflibercept to FOLFIRI3-aflibercept.

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P- Reviewer: Tsushima T, Velenik V, Zhang J **S- Editor**: Ji FF
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