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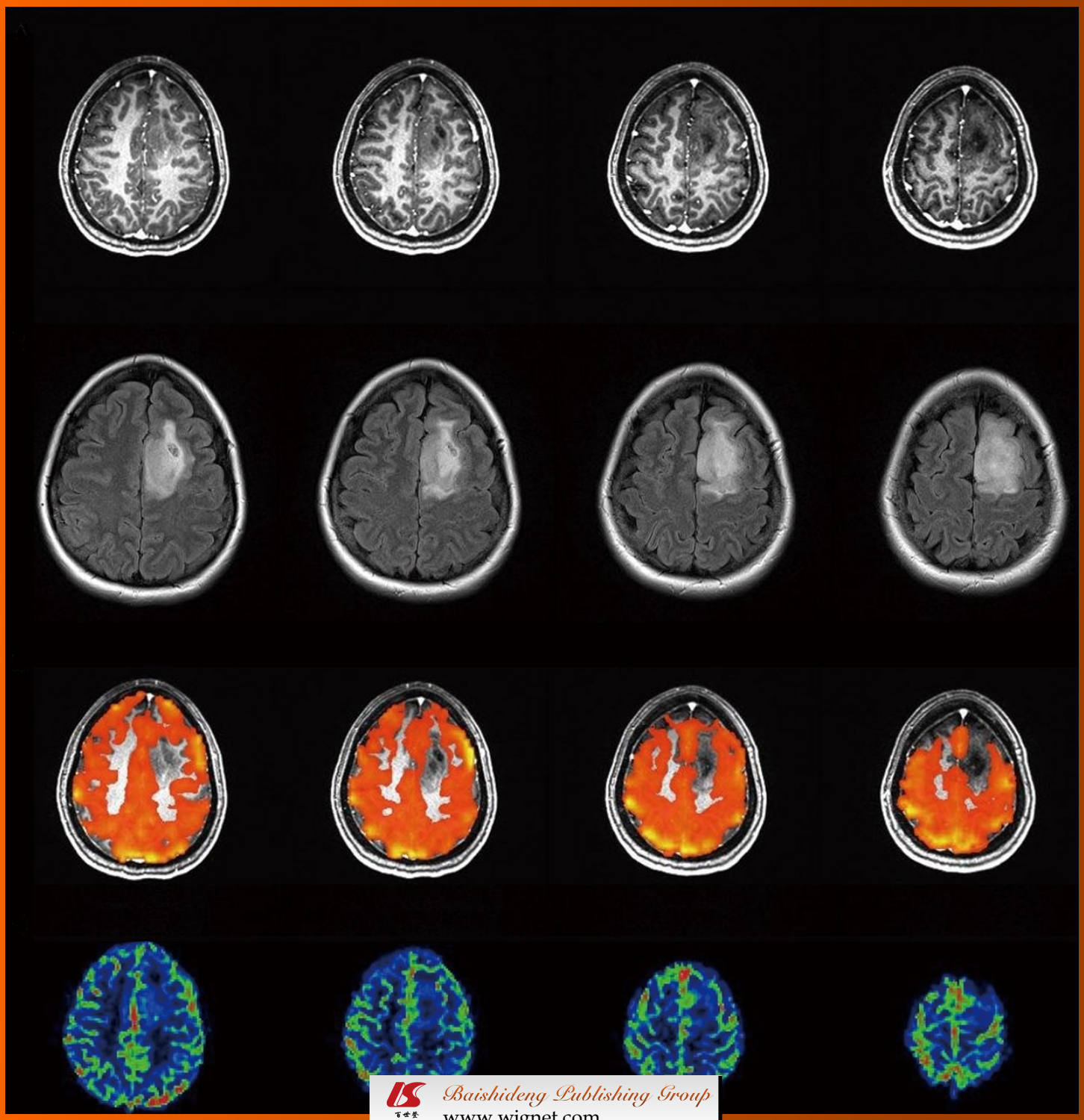

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EDITORIAL

Locally advanced nasopharyngeal carcinoma: Current and emerging treatment strategies

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Abstract

Although nasopharyngeal carcinoma (NPC) is a widespread malignant tumor, it is particularly frequent in Southeast Asia. Although T1 tumors can be effectively controlled with exclusive radiotherapy, this treatment modality is insufficient for most NPC patients, who present with locally advanced disease at diagnosis. In fact, for stages ranging from T2b N0 to T4 N3, definitive scientific evidence supports the use of concurrent platinum-based chemotherapy with standard external beam radiotherapy. This treatment approach has shown a statistically significant advantage in terms of overall survival, with respect to radiotherapy alone. Several trials have also investigated the use of neoadjuvant and adjuvant chemotherapy in combination with radiotherapy or chemo-radiotherapy. Platinum compounds, anthracyclines and taxanes are among the chemotherapy agents employed. This review focuses on the clinical results obtained in the field of adjuvant/concurrent/neoadjuvant chemotherapy for locally advanced NPC, for which exclusive concurrent chemo-radiotherapy currently represents the standard treatment approach.

INTRODUCTION

Nasopharyngeal carcinoma (NPC), a malignant tumor originating from the epithelium of the nasopharynx, is particularly frequent in Southeast Asia and can be divided into three different histological types, that is, non-keratinizing squamous cell carcinoma, keratinizing squamous cell carcinoma and undifferentiated carcinoma[1]. At diagnosis, most NPC patients have locally advanced disease, which includes stages ranging from T2b N0 to T4 N3 (Table 1).

Radiotherapy (RT) can control early stage NPC effectively, yielding an excellent 90%-95% 5-year local control rate in clinical trials. However, radiotherapy alone is not the optimal treatment for patients with locally advanced disease, which is the most frequent clinical presentation at diagnosis, since it yields an unsatisfactory 5-year survival rate of about 50%[2]. For this reason, concurrent platinum-based chemotherapy and radiotherapy has become the standard treatment for locally advanced NPC. While in early stage NPC (T1-2a N0), the addition of chemotherapy to standard radiotherapy has not provided

Table 1 Standard approach to nasopharyngeal carcinoma

Stage	Denomination	Gold standard therapy
T1-2a N0 M0	Early stage	- IMRT alone - Conventional RT alone
From T2b N0 M0 to T4b N3 M0 also every T N2/3 M0	Locally advanced	- Neoadjuvant platinum-based CT followed by IMRT or CCRT (platinum-based) - Concurrent cDDP and RT
Every T every N M1	Metastatic	- Exclusive CT

RT: Radiotherapy; IMRT: Intensity modulated RT; CCRT: Concurrent chemoradiotherapy; CT: Comptued tomography.

a survival advantage in clinical trials[3], a clear superiority has emerged for concurrent chemoradiotherapy when compared to RT alone in patients with locally advanced disease[4-5].

In locally advanced NPC patients, there are presently few data regarding the use of neoadjuvant/adjuvant chemotherapy, as an alternative to concurrent chemoradiotherapy. The role of neoadjuvant chemotherapy before RT or concurrent chemoradiotherapy is a matter of great interest. In fact, induction chemotherapy is an effective way to control subclinical metastatic foci, especially in patients with lymph node metastasis. Moreover, in some patients with large tumors infiltrating the brain stem, it is often difficult to deliver the total required dose to the clinical target volume (CTV) with preservation of critical tissues. Neoadjuvant chemotherapy is often able to provide objective responses in tumor lesions, which offers the possibility to shrink the CTV and reduce toxicity[6].

Retrospective studies that used RT alone for NPC indicated that local control was closely linked to the radiation dose delivered to target tissues[7-8]. Intensity modulated RT (IMRT) is a special type of conformal RT that creates a high dose volume that is precisely shaped around the target volume in order to minimize the radiation dose delivered to surrounding healthy tissues. Investigators compared dosimetric plans of IMRT with conventional RT techniques and concluded that IMRT provided improved tumor coverage and preservation of normal tissues. The proximity of the nasopharynx to critical normal tissues, such as the brainstem and optic structures, makes it challenging for radiotherapists to deliver the optimal radiation dose to the tumor using conventional conformal RT, and underdosing of affected areas is often necessary to preserve healthy tissues. IMRT for locally advanced NPC spares critical portions of the brain stem and of the parotid glands, avoiding neurologic toxicity and permanent xerostomia, respectively. While IMRT has completely replaced conventional conformal RT and has become the standard practice for early stage NPC, its role in the locally advanced setting is not yet well defined[9].

Intracavitary brachytherapy may be used in patients with residual mass after exclusive upfront radiotherapy, especially in the case of a T2b tumor (parapharyngeal infiltration) at initial diagnosis[10]. The combination of external beam RT followed by endocavitary brachytherapy may play an important role in patients with T2b disease. In this review, the treatment of patients with locally advanced NPC is reviewed and discussed, with a special focus on novel experimental therapeutic options.

ROLE OF EXCLUSIVE CONCURRENT CHEMORADIOTHERAPY

In several phase Ⅲ trials, radiotherapy with concurrent platinum-based chemotherapy has been compared to standard external beam radiotherapy alone in patients with locally advanced NPC. Concurrent chemo-radiotherapy has shown a statistically significant advantage in terms of survival and response rate when compared with radiotherapy alone, but at the expense of more severe toxicity, mainly mucositis and bone marrow suppression[4,5,11]. Results of a large meta analysis carried out by Zhang *et al*[12] which included 1608 patients enrolled in seven studies confirmed the superiority of concurrent chemo-radiotherapy with respect to RT alone. Of note, this meta-analysis was the first to include studies conducted in endemic areas only.

Another meta-analysis included 18 trials enrolling a total of 1993 patients from China. A comparison between concurrent chemo-radiotherapy and RT alone showed that concurrent chemo-radiotherapy was able to obtain a 3-year overall survival rate of 68.5%, compared with 56.4% in the RT alone arm[13].

More recently, the association of cisplatin and paclitaxel given concurrently with standard radiotherapy was evaluated in a phase Ⅱ trial. Thirty-one patients with locally advanced NPC received three-weekly 120 mg/m2 of paclitaxel and 75 mg/m2 of cisplatin concurrently with standard 70 Gy external beam radiotherapy. Three-year overall survival rate was 83.9% and the main grade 3/4 toxicity was neutropenia, reported in 12.9% of patients[14]. Another way of improving the effectiveness of standard concurrent chemo-radiotherapy may be to modify the radiotherapy scheme. In a phase Ⅱ trial, Jian *et al*[15] investigated the activity of hyperfractionated radiotherapy and concomitant platinum-based chemotherapy. As a result, three-year overall survival rate was 72%, with 73% of patients showing grade 3 mucositis, 31% of patients experiencing severe weight loss and 15% requiring a feeding tube. In view of such an unfavorable toxicity profile, further investigation in this direction does not seem justified.

IMRT is widely employed as an alternative to conventional RT in NPC patients with stage Ⅰ-Ⅱ disease, but its role in association with chemotherapy is still unknown. Lu *et al*[16] evaluated the feasibility and efficacy of a weekly cisplatin (40 mg/m^2/wk) regimen given concurrently with definitive IMRT in twenty-one locally advanced NPC patients, obtaining a good safety profile and an excellent one-year overall survival of 95.5%[17]. In another similar trial, the association of three-weekly cisplatin and weekly cetuximab was employed together with standard IMRT

Table 2 Chemo-radiation trials

Trial	Phase	Pts	Study design	Main end-point	Results
Lin JC *et al*[4]	Ⅲ	284	Exclusive RT alone *vs* cDDP-5FU + RT	5-year DFS	Experimental arm better ($P < 0.0012$)
Chan AT *et al*[5]	Ⅲ	350	Exclusive RT alone *vs* cDDP-5FU + RT	2-year PFS	Experimental arm better ($P < 0.016$)
Zhang L *et al*[12]	Ⅲ(m)	1608	Exclusive RT alone *vs* cDDP based CT + RT	5-year OS	Experimental arm better ($P < 0.001$)
Yang AK *et al*[13]	Ⅲ(m)	1993	Exclusive RT alone *vs* cDDP based CT + RT	5-year OS	Experimental arm better ($P < 0.05$)
Lu H *et al*[17]	Ⅱ	22	IMRT + cDDP	1 year OS	96%
Ekenel M *et al*[34]	Ⅱ	100	IMRT+ cDDP-Cet	ORR	100%

RT: Radiotherapy; IMRT: Intensity modulated RT; CT: Comptued tomography; ORR: Overall response rate; DFS: Disease-free survival.

in 100 locally advanced NPC patients. The complete + partial response rate achieved was 100% and the toxicity profile was very low, except for a 64% rate of grade 2 acneiform rash. Clinical studies assessing the efficacy and activity of exclusive concurrent chemo-radiotherapy in locally advanced NPC patients are shown in Table 2.

ROLE OF ADJUVANT CHEMOTHERAPY FOLLOWING CONCURRENT CHEMO-RADIOTHERAPY

The Intergroup-0099 was the first randomized trial to compare concurrent chemo-radiotherapy followed by adjuvant chemotherapy with RT alone[18]. In this study, concurrent chemo-radiotherapy consisted of cisplatin (100 mg/m^2 every 21 d) for three cycles, followed by adjuvant cisplatin (80 mg/m^2 on day 1) and 5-fluorouracil (1000 mg/m^2 on days 1-4 every 4 wk). A clear and statistically significant advantage in the chemo-radiation arm was seen in terms of overall survival, disease-free-survival, locoregional failure rate and time to distant metastases. However, these encouraging results did not translate into a change in clinical practice for many Asian oncologists, in view of the high rate of well differentiated carcinomas enrolled in both treatment arms (about 25%) which does not reflect common clinical practice. Furthermore, a particularly low compliance was reported, with only 55% undergoing adjuvant treatment and a particularly poor survival observed in the RT-alone arm.

A comparison between concurrent chemo-radiotherapy followed by adjuvant chemotherapy and RT alone was performed in 316 locally advanced NPC patients enrolled in a phase Ⅲ trial conducted by Chen *et al*[19]. Patients were assigned to receive concurrent chemo-radiotherapy, consisting of a standard radiation dose of 70 Gy plus cisplatin followed by three adjuvant cycles of cisplatin and 5-fluorouracil, or RT alone. Concurrent chemo-radiotherapy plus adjuvant chemotherapy yielded better results with respect to RT alone in terms of survival and activity, at the cost of higher toxicity. Similar results were seen in another phase Ⅲ trial enrolling only non-keratinizing locally advanced NPC patients randomized to concurrent chemo-radiotherapy followed by adjuvant cisplatin and 5-fluorouracil or to RT alone. In this trial, concurrent chemo-radiotherapy was superior in terms of efficacy but also more toxic than RT alone[20].

Park *et al*[21] carried out a retrospective analysis in forty-three locally advanced NPC patients treated with concurrent chemo-radiotherapy using cisplatin and 5-fluorouracil followed by adjuvant chemotherapy consisting of three cycles of cisplatin, epirubicin and bleomycin. The overall response rate (ORR) was 95% after concurrent chemo-radiotherapy and 100% after adjuvant therapy. The main toxicities observed were grade 3/4 neutropenia and mucositis occurring during concurrent chemo-radiotherapy.

In a prospective phase Ⅱ trial conducted by Hu *et al*[22] fifty-four patients were treated with concomitant weekly paclitaxel and external beam radiation therapy (concurrent chemo-radiotherapy) followed by three cycles of cisplatin (30 mg/m^2 on days 1-3) and paclitaxel (135 mg/m^2 on day 1), both given every three weeks. An excellent 100% ORR was obtained after the entire treatment with a complete response (CR) rate of 85%. An acceptable toxicity profile was seen with no grade 3/4 side effects.

In view of the conflicting results reported on the role of adjuvant chemotherapy after concurrent chemo-radiotherapy, it is presently unclear whether the addition of adjuvant therapy may improve the efficacy of concurrent chemo-radiotherapy. Interestingly, a combined analysis of two large studies (NPC-9901 and the NPC-9902) revealed that the dose of cisplatin during the concurrent phase of concurrent chemo-radiotherapy had a significant impact on locoregional control, while additional adjuvant chemotherapy with a fluorouracil-containing combination contributed to improving distant control. Table 3 shows the results of clinical trials assessing the efficacy and/or activity of adjuvant chemotherapy following concurrent chemo-radiotherapy.

ROLE OF NEOADJUVANT CHEMOTHERAPY FOLLOWED BY CONCURRENT CHEMO-RADIOTHERAPY

The role of neoadjuvant chemotherapy followed by concurrent chemo-radiotherapy or RT is a matter of outstanding interest. Several clinical phase Ⅲ trials from Western countries have proved that induction chemotherapy based on the administration of cisplatin, 5-fluorouracil and taxanes, may significantly improve treatment outcomes in patients with squamous cell carcinoma of the head and neck. An interesting approach may be to employ the same chemotherapy or a similar regimen in locally advanced NPC patients. Amro *et al*[23] treated 110

Table 3 Adjuvant chemotherapy trials

Trial	Phase	Pts	Study design	Main end-point	Results
Al-Sarraf M *et al*[18]	Ⅲ	147	Exclusive RT alone *vs* CCRT followed by cDDP-5FU	3-year PFS	Experimental arm better ($P < 0.01$)
Chen Y *et al*[19]	Ⅲ	316	Exclusive RT alone *vs* CCRT followed by cDDP-5FU	2-year OS	Experimental arm better ($P < 0.003$)
Lee AW *et al*[20]	Ⅲ	348	Exclusive RT alone *vs* CCRT followed by cDDP-5FU	5-year PFS	Experimental arm better ($P < 0.035$)
Park KH *et al*[21]	Ⅱ	43	cDDP-5-FU + RT followed by cDDP-Epi-Ble CT	ORR	100%
Hu W *et al*[22]	Ⅱ	54	w Pac + RT followed by cDDP-Pac CT	ORR	100%
Leung TW *et al*[10]	Ⅱ	48	HFRT + cDDP based CT followed by cDDP-5FU CT	3-year DFS	71%

RT: Radiotherapy; CT: Comptued tomography; ORR: Overall response rate; CCRT: Concurrent chemoradiotherapy; DFS: Disease-free survival.

patients with induction cisplatin and epirubicin followed by a radical course of radiotherapy with three cycles of concurrent cisplatin, and obtained encouraging results in terms of safety and effectiveness. Italian investigators used the same treatment schedule in 40 patients and obtained an overall response rate of 100% and a 5-year disease-free survival of 77%[24]. In another Italian phase Ⅱ study, Ferrari *et al*[25] treated thirty-four patients with three cycles of neoadjuvant cisplatin and 5-fluorouracil followed by concurrent cisplatin and RT. As a result, the overall response rate obtained was a satisfactory 85.3% and the 3-year overall survival rate was 80%.

In the last five years, taxanes have been employed in several phase Ⅱ and Ⅲ clinical trials in patients with squamous cell carcinoma of the head and neck, showing a good activity and manageable toxicity profile. Lu *et al*[26] carried out a trial to compare two different schedules of induction chemotherapy, namely carboplatin-5-fluorouracil (CF) *vs* docetaxel-carboplatin (TC). Fifty-eight patients with locally advanced NPC were enrolled and randomized to receive CF or TC induction chemotherapy, both followed by concurrent carboplatin and RT. There was no significant difference in terms of response rate and 1-year survival rate. More grade 3/4 neutropenia events were reported in the TC group than in the CF group, whereas less grade 3/4 thrombocytopenia and emesis occurred with the TC regimen than with the CF regimen. An egyptian study enrolled thirty-six patients who were treated with three cycles of induction paclitaxel (175 mg/m^2) and cisplatin (80 mg/m^2) given every three weeks, followed by concomitant cisplatin-radiotherapy. The overall response rate after the entire treatment schedule was 89% and the 3-year overall survival was 68%. The main toxicity encountered was grade 3/4 neutropenia which was observed in 25% of patients[27]. Hui *et al*[28] published the results of a randomized phase II trial in which stage III-IVb NPC patients, not previously treated, were randomly assigned to receive either neoadjuvant docetaxel and cisplatin for two cycles followed by concurrent chemo-radiotherapy, or concurrent chemo-radiotherapy alone. A positive impact on survival was observed, since the 3-year overall survival for the neoadjuvant *versus* the control arm was 94.1% *versus* 67.7% ($P = 0.012$). Bossi *et al*[29] recently presented data of a study on docetaxel, cisplatin and 5-fluorouracil as induction chemotherapy followed by concomitant cisplatin/RT. After completion of treatment, the ORR was 98%, with a complete response rate of 70%. Other authors showed that the same combination had similar results with a 93% response rate and a median time to progression of 39 months[30]. In a phase II study, induction docetaxel, cisplatin and capecitabine followed by chemo-radiation was tested in 40 patients, and resulted in an ORR of 98% and a complete response rate of 48%[31]. In a phase Ⅱ clinical study, Bae *et al*[32] treated thirty-three patients with induction cisplatin (70 mg/m^2), 5-fluorouracil (1000 mg/m^2 in i.c of 4 d) and docetaxel (75 mg/m^2) followed by cisplatin (100 mg/m^2) and RT. Twenty-seven patients achieved a partial response and five patients a complete response. An excellent ORR of 98% was achieved and the three-year overall survival rate was 86.1%. Nevertheless, a 72.7% rate of grade 2/3 neutropenia and a 9.1% rate of febrile neutropenia were reported. Xie *et al*[33] administered induction cisplatin (80 mg/m^2) and docetaxel (70 mg/m^2) to fifty-seven patients and randomized them to receive either concomitant RT and single agent cisplatin (80 mg/m^2) or concomitant cisplatin (80 mg/m^2) and docetaxel (60 mg/m^2) with RT. After completion of treatment, the complete response rates were very similar in both treatment arms (about 93%), but the occurrence of grade 3/4 neutropenia was significantly higher in the concomitant docetaxel, cisplatin and RT group ($P > 0.05$). In a recent phase Ⅱ clinical study, fifty-nine locally advanced NPC patients were treated with neoadjuvant cisplatin (75 mg/m^2), docetaxel (75 mg/m^2) and 5-fluorouracil (500 mg/m^2 on days 1-5 in i.c) for three cycles, followed by concomitant weekly cisplatin (40 mg/m^2) and conventional RT or IMRT. The overall response rate three months after RT was 90.2% and the 1-year overall survival was 100%. The rate of grade 3/4 myelosuppression during induction CT was 55.9% and the corresponding rate during concomitant chemotherapy and RT was 11.9%[6]. More recently, Ekenel *et al*[34] published the preliminary results of a phase Ⅱ trial in which patients with locally advanced NPC received induction cisplatin (75 mg/m^2) and docetaxel (75 mg/m^2) for three cycles, followed by definitive RT and concomitant cisplatin (100 mg/m^2). Fifty-nine patients were evaluable and the ORR obtained after RT was 95%. Three-year overall survival was 93% and the treatment was generally well tolerated with a 10% rate of grade 3/4

Table 4 Neoadjuvant chemotherapy trials

Trial	Phase	Pts	Study design	Main end-point	Results
Al-Amro A *et al*[23]	Ⅱ	110	Neo cDDP-Epi and followed by cDDP + RT	ORR	100%
Airoldi M *et al*[24]	Ⅱ	30	Neo cbdca-Pac followed by RT + cbdca-Pac	ORR	87%
Ferrari D *et al*[25]	Ⅱ	34	Neo cDDP-5FU followed by RT + cDDP	ORR	85.3%
Lu X *et al*[26]	Ⅱ	58	Neo cbdca-Tax followed by cbdca + RT (arm A) *vs* neo cbdca-5FU followed by cbdca + RT (armB)	1-year DFS	no difference between arm A and B
Mosatafa E *et al*[27]	Ⅱ	36	Neo cDDP-Pac followed by cDDP-RT	ORR	89%
Hui EP *et al*[28]	Ⅱ	65	Neo cDDP-Tax followed by cDDP + RT (arm A) *vs* cDDP + RT (arm B)	3-year OS	Arm A better than arm B ($P < 0.012$)
Bossi P *et al*[29]	Ⅱ	45	Neo cDDP-Tax-5FU followed by cDDP + RT	ORR	98%
Cho S *et al*[30]	Ⅱ	19	Neo cDDP-Tax-5FU followed by cDDP + RT	ORR	93%
Bae WK *et al*[32]	Ⅱ	33	Neo cDDP-Tax-5FU followed by cDDP + RT	ORR	99%
Kong L *et al*[6]	Ⅱ	52	Neo cDDP-Tax-5FU followed by cDDP + RT	ORR	90.2%
Ekenel M *et al*[34]	Ⅱ	59	Neo cDDP-Tax followed by cDDP + RT	ORR	95%
Lin S *et al*[35]	Ⅱ	370	Neo cDDP based CT followed by IMRT	3-year OS	90%

RT: Radiotherapy; IMRT: Intensity modulated RT; CT: Comptued tomography; ORR: Overall response rate; DFS: Disease-free survival.

hematologic toxicity.

In the last three years, a significant effort has been made to incorporate IMRT in treatment protocols for locally advanced NPC. In a phase Ⅱ study, Lin *et al*[35] treated a total of 370 patients with locally advanced NPC, with stages ranging from Ⅱb to Ⅳ, with induction cisplatin-based chemotherapy followed by exclusive IMRT or the association of IMRT and concomitant cisplatin. Drugs more frequently used in combination with cisplatin were paclitaxel and 5-fluorouracil. With a median follow-up of 31 mo, the three-year disease-free-survival and overall survival were 81% and 89%, respectively. A subgroup analysis revealed that concurrent chemotherapy provided no significant benefit to IMRT but was responsible for higher rates of grade 3/4 toxicities[35]. A recent Italian phase Ⅱ trial carried out by Palazzi *et al*[36] enrolled 87 patients with locally advanced NPC and treated them with either conventional RT or with IMRT. Of these patients, 26% received only concurrent cisplatin and the other 74% received both induction and concurrent CT. Three-year disease-free survival (DFS) and overall survival were 82% and 90%, respectively. Interestingly, a multivariate analysis revealed that histology, N-stage, RT-technique and total dose of RT had the strongest independent impact on DFS.

Further clinical trials assessing activity, efficacy and toxicity of the combination of induction taxane-based chemotherapy followed by exclusive IMRT in locally advanced NPC are warranted. At the present time, this treatment strategy is recommended only in experienced centers. Table 4 shows clinical trials assessing the activity and efficacy of neoadjuvant chemotherapy followed by radiation or chemo-radiation.

CONCLUSION

This review reported in detail the available clinical data regarding the use of chemotherapy in combination with radiotherapy for locally advanced NPC. Although several cytotoxic agents have been used both in the neoadjuvant and adjuvant setting with promising results, exclusive concurrent chemo-radiotherapy remains the recommended approach at the present time, as additional evidence is required to support the use of chemotherapy in the adjuvant/neoadjuvant setting.

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IL-6/IL-6R as a potential key signaling pathway in prostate cancer development

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Abstract

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in prostate regulation and in prostate cancer (PC) development/progression. IL-6 acts as a paracrine and autocrine growth stimulator in benign and tumor prostate cells. The levels of IL-6 and respective receptors are increased during prostate carcinogenesis and tumor progression. Several studies reported that increased serum and plasma IL-6 and soluble interleukin-6 receptor levels are associated with aggressiveness of the disease and are associated with a poor prognosis in PC patients. In PC treatment, patients diagnosed with advanced stages are frequently submitted to hormonal castration, although most patients will eventually fail this therapy and die from recurrent castration-resistant prostate cancer (CRPC). Therefore, it is important to understand the mechanisms involved in CRPC. Several pathways have been proposed to be involved in CRPC development, and their understanding will improve the way to more effective therapies. In fact, the prostate is known to be dependent, not exclusively, on androgens, but also on growth factors and cytokines. The signaling pathway mediated by IL-6 may be an alternative pathway in the CRPC phenotype acquisition and cancer progression, under androgen deprivation conditions. The principal goal of this review is to evaluate the role of IL-6 pathway signaling in human PC development and progression and discuss the interaction of this pathway with the androgen recepto pathway. Furthermore, we intend to evaluate the inclusion of IL-6 and its receptor levels as a putative new class of tumor biomarkers. The IL-6/IL-6R signaling pathway may be included as a putative molecular marker for aggressiveness in PC and it may be able to maintain tumor growth through the AR pathway under androgen-deprivation conditions. The importance of the IL-6/IL-6R pathway in regulation of PC cells makes it a good candidate for targeted therapy.

Key words: Androgen receptor; Castration-resistant prostate cancer; Hormonal castration; Inteuleukin-6; Inteuleukin-6 receptor; Prostate cancer; Tumor biomarker

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INTRODUCTION

Prostate cancer (PC) is the most common cancer among men in Western populations[1]. Ethnicity, advanced age and family history are well known risk factors for this disease[1]. Furthermore, circulating androgen levels, chronic prostate inflammation and obesity are also risk factors frequently described in the literature[2,3]. Currently, prostate-specific antigen (PSA) is the putative biomarker for PC screening. Two consecutive rises in PSA value over 0.5 ng/mL or one single value ⩾ 4 ng/mL are indications for biopsy[4]. However, although PSA testing has high sensibility, its specificity is rather low, causing clinicians to have doubts with regard to biopsying, since increased false-positive rates, overdiagnosis and overtreatment have been reported to be associated with PSA testing[5-8]. Therefore, novel biomarkers are needed to improve identification of men at risk of having PC and to predict the natural behavior of the prostate tumor. The use of more sensitive and specific biomarkers will be an appropriate strategy for disease diagnosis, disease staging, disease prognosis, predicting and monitoring clinical response to therapy. In consequence of the high heterogeneity of PC, it is relevant to study molecular and cellular pathways involved in its development and progression to identify key genes and molecules implicated in different stages of the disease[9].

In several diseases, a deregulation of cytokine levels can be observed. Numerous pro-inflammatory cytokines play an important role in the pathogenesis/carcinogenesis of many cancers. One of the most important cytokine associated with inflammation, interleukin-6 (IL-6), will be discussed in this manuscript.

Recent evidence suggests that the presence of inflammatory factors and cytokines at the tumor site results in tumor cell survival, proliferation, invasion and metastasis[10]. The expression and function of pro-inflammatory cytokines in PC have been extensively investigated because of their role in the regulation of proliferation, apoptosis, migration, invasion, and angiogenesis[11]. Recent studies have focused on the role of cytokines, including IL-6, in the etiology and progression of PC[12-16]. Elevated levels of IL-6 in men with local PC and advanced disease made IL-6 a candidate biomarker for PC development and progression[17].

This review is a summary of several studies outlining the potential role of the IL-6 signaling pathway in human PC development and progression, an interesting area of scientific and clinical research.

IL-6 AND RECEPTORS IN CANCER

IL-6 is involved in the regulation of various cellular functions, among them proliferation, apoptosis, angiogenesis, differentiation and regulation of immune response[12]. This protein is a pleiotropic cytokine synthesized by different cell types, such as B and T-cells, macrophages, monocytes, fibroblasts, endothelial and mesothelial cells, keratinocytes, mast cells, stromal cells, some nerve cells and certain tumor cells[18]. Adipose tissue is another main source of IL-6[19].

The biological activity of IL-6 is initiated when the cytokine binds to a receptor complex: an 80-kDa component receptor, non-signaling α-receptor subunit (IL-6R or 80gp) and two signal-transduction components of 130 kDa (gp130) (Figure 1)[20,21]. IL-6R is expressed only by hepatocytes, neutrophils, monocytes/macrophages and some lymphocytes, while gp130 is expressed by all body cells[22]. Dimerization of IL-6/IL-6R/gp130 lead to the initiation of intracellular signaling, through Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT), Mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/Akt kinase (PI3-K/AKT) pathways[23] and consequently activate the expression of different genes with crucial roles in inflammation and cancer development. This mechanism is the classical signaling pathway[24]. When IL-6 binds to the receptor, STAT3 is activated in a JAK-dependent manner that leads to increased receptor activator of nuclear factor κB ligand (RANKL) expression. IL-6 may also activate AKT *via* increased JAK-dependent PI3K activity and results in cell survival and anti-apoptosis signaling. Concomitantly, increased MAPK activity downstream of JAK activation can lead to up-regulated cell growth, proliferation, and mitosis (Figure 1)[25].

Some cells express lower levels of IL-6 transmembrane receptor, in this case IL-6 can bind to a soluble form of the IL-6R (sIL-6R). Then, through a process denominated trans-signaling, the IL-6/sIL-6R complex binds to gp130 and subsequently signal transduction pathways are activated (Figure 1)[24,26]. Due to the fact that the sIL-6R lacks a membrane signaling domain, there appears to be significant differences in the intracellular signaling pathways. While IL-6 trans-signaling also leads to phosphorylation and activation of STAT3, increased cell survival, proliferation, and mitosis occurs in an AKT- and MAPK-independent manner. The exact mechanisms for IL-6 trans-signaling leading to increased cell survival, proliferation, and mitosis are not yet known (Figure 1)[25].

In humans, sIL-6R can be generated by proteolytic cleavage (90%) of the transmembrane receptor mediated by metalloproteinases such as a disintegrin and metalloproteinase 10 and 17[27,28] or by an alternative mRNA splicing (10%)[29].

A soluble form of gp130 (sgp130) is present at con-

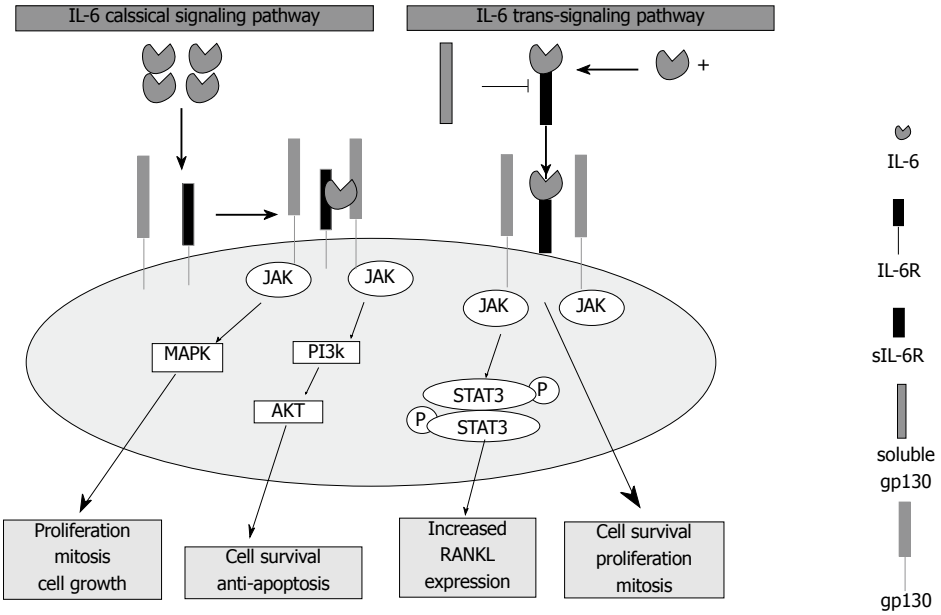

Figure 1 Schematic of IL-6 classical and trans-signaling pathway. IL-6: Interleukin 6; JAK: Janus kinase; MAPK: Mitogen-activated protein kinase; PI3K; Phosphatidylinositol 3-kinase; RANKL: Receptor activator of nuclear factor κB ligand; sIL-6R: Soluble IL-6 receptor; STAT3: Signal transducer and activator of transcription 3.

centrations between 100-400 ng/mL (1-4 nmol/L) in healthy human serum[30]. Jostock *et al*[31] reported that IL-6 alone does not interact with sgp130. Thus, signaling *via* the membrane-bound IL-6R is not inhibited by sgp130[31]. Based on their results, it was suggested that endogenous sgp130 may be a natural antagonist of the IL-6/sIL-6R complex *in vivo*, probably to prevent systemic IL-6 trans-signaling during inflammatory diseases[31]. Sgp130 inhibits the activity of the IL-6/sIL-6R complex and is in competition with gp130 for this complex without interfering with the classical IL-6 signaling pathway (Figure 1)[31-33]. A schematic of IL-6 classical and trans-signaling pathway is shown in Figure 1. In classical signaling, IL-6 binds first to the membrane-bound non-signaling IL-6R. After recruitment of two gp130 molecules the complex is formed and signal transduction is induced; in trans-signaling, IL-6 binds to the sIL-6R, which is generated by ectodomain shedding of the surface receptor or alternative splicing. The IL-6/sIL-6R can bind to both membrane-bound or sgp130 and a molar excess of sgp130 leads to competitive inhibition of the IL-6/sIL-6R response. Sgp130 has no access to the IL-6/IL-6R complex of the classical signaling pathway. Figure 1 was adapted from[25,34,35].

IL-6 may have a crucial role in the growth and differentiation of malignant tumors. IL-6 has multiple effects on tumor progression, some are the result of autocrine activity on tumor cells and others are a consequence of paracrine action on normal cells in the tumor microenvironment, particularly osteoblasts, osteoclasts, endothelial cells and immune cells[36]. For example, tumor cells from prostate, breast and colon cancer produce large amounts of IL-6 and express its receptors, IL-6R (gp80) and gp130, allowing them to respond in an autocrine manner to IL-6[28]. Moreover, multiple myeloma and neuroblastoma cells do not produce IL-6R but express IL-6. These cells respond in a paracrine manner to IL-6 present in the tumor microenvironment[36].

IL-6 can also modulate the metastatic process. High IL-6 expression in specific organs such as lung, liver or brain may attract circulating tumor cells to these organs, promoting the development of metastases[28]. Recently, it was demonstrated that the production of IL-6 and IL-8 in the primary tumor was responsible for the recruitment of circulating tumor cells to the primary site, resulting in a process called self-seeding which would lead to rapid tumor growth, angiogenesis, as well as stromal cell recruitment[29].

An elevated serum IL-6 level has been correlated with adverse prognosis in patients with several different types of cancer, such as multiple myeloma[37], lymphoma[38], ovarian cancer[39-41], PC[42], metastatic renal carcinoma[43], lung cancer[44], and breast cancer[45,46]. Hence, the interest in using serum IL-6 as a specific prognostic factor for PC and breast cancer has increased[45,47].

On the other hand, trans-signaling through the IL-6/sIL-6R complex has been shown to have an important role in inflammatory diseases and in the development and progression of various malignant tumors[34].

IL-6 trans-signaling has become a new area of research, and it was shown that sIL-6R is produced by various cancer cells, the serum concentration of sIL-6R is associated with decreased survival and increased aggressiveness of metastases in breast, prostate and colorectal cancers[48-50]. The source of sIL-6R is not known, but it is shed by inflammatory cells including neutrophils, monocytes/macrophages and T cells[51,52]. On the other hand, several tumor cells can shed IL-6R or produce it as a result of alternative splicing[53]. Some data suggest that IL-6 trans-signaling causes various effects that promote cancer metastases including, increased detachment, proliferation

and migration[54].

IL-6 has emerged as an important cytokine in the tumor microenvironment, which may contribute to the development and progression of various malignant tumors. Currently, little is known about the IL-6 trans-signaling pathway, and we suggest more studies to improve knowledge on the sIL-6R as a potential therapeutic target.

IL-6 PATHWAY IN PROSTATE CANCER

IL-6 serum levels and its receptors in prostate cancer patients

Involvement of IL-6 in PC development and progression has been suggested by several studies through increased IL-6 and sIL-6R levels during PC carcinogenesis and progression[12-14]. Baillargeon *et al*[15] suggested that serum and plasma IL-6 and sIL-6R levels are associated with progression and poor prognostic in PC patients.

Clinical observations have demonstrated increased IL-6 levels in plasma and serum from patients with CRPC[55,56], metastatic PC[42,48,57-59], biochemical recurrence[42] and poorer overall survival[15,60] compared to patients with earlier stages of the disease and healthy individuals.

Other studies support these findings and have shown that IL-6 can be correlated with the extension of disease, tumor size and bone metastases in PC[48,61].

Akimoto *et al*[57] found that serum levels of IL-6 were significantly higher in PC patients with bone metastasis than in PC patients without bone metastasis. These results suggested that serum levels of IL-6 were closely related to the metastatic burden of osseous tissue in PC patients[57]. Adler *et al*[58] reported that patients with metastatic PC had significantly elevated IL-6 levels when compared with those in other PC groups as well as controls. Of the 9 patients with distant metastatic disease (M1), 7 had elevated serum IL-6 levels. However, mean serum IL-6 was similar in patients with organ confined cancer (pT2) and in those with nodal metastases (N1). Eight of the 12 patients with N1 disease had decreased serum IL-6 compared to the pT2 group.

In another study, patients with metastatic PC were compared to patients with localized PC, and significant differences in IL-6 levels among the groups were observed. IL-6 levels were significantly higher in patients with metastatic disease. Patients with lymph node metastases or bone metastases had similar IL-6 levels. Serum IL-6 was significantly elevated in patients with Gleason score > 6[59]. Shariat *et al*[48] observed that the preoperative IL-6 and IL-6sR levels were elevated in patients with a final Gleason sum of 7 or greater. They reported that neither IL-6 nor IL-6sR were predictors of organ-confined disease. The mean preoperative IL-6 and sIL-6R plasma levels were higher in patients with aggressive disease than in those with non aggressive phenotype. IL-6 and sIL-6R plasma levels in patients with PC metastatic to bone were higher than those in patients with metastases in regional lymph nodes and these, in turn, were higher than those in prostatectomy patients and healthy subjects. However, the IL-6 and IL-6sR levels were not different between the prostatectomy patients and the healthy subjects.

Regarding patients with CRPC, Drachenberg *et al*[55] found that serum levels of IL-6 were significantly elevated in patients with clinically evident CRPC compared to normal controls, patients with BPH, prostatitis, and localized or recurrent disease. Wise *et al*[56] observed elevated levels of the anti-inflammatory cytokine, IL-6, in CRPC patients when compared to patients with CSPC.

The results obtained by George *et al*[60] showed that IL-6 levels were higher in patients with metastatic disease and these patients had worse survival when compared with patients who had low IL-6 levels. An analysis by Nakashima *et al*[15] verified a significantly shorter survival in patients with elevated IL-6 levels, PSA serum levels and aggressive disease.

Additionally, Shariat's[48,62,63] group demonstrated that preoperative plasma levels of IL-6, sIL-6R and transforming growth factor-beta1 (TGF-β1) predicted biochemical recurrence after surgery or radical prostatectomy, suggesting an association with occult metastatic disease at the time of radical prostatectomy. The results of Alcover *et al*[64] are in agreement with Shariat's group, as they observed that IL-6 predicts biochemical recurrence following radical prostatectomy. More recently, a predictive model was proposed that included only sIL-6R[65]. Kattan *et al*[66] developed and validated a prognostic model that added plasma sIL-6R and TGF-β1 as standard clinical predictors for biochemical recurrence. However, Baillargeon *et al*[16] found that serum IL-6 levels were not associated with PC. According to Finley *et al*[67] the differences among various studies are a consequence of systemic cytokine levels detected in peripheral blood that may not reflect local concentrations in the tumor microenvironment.

IL-6 levels can also be elevated in obese individuals[68]. The relationship between obesity, circulating IL-6 and PC may help to understand the role of this molecule and how it contributes to the molecular basis for the association between obesity and PC[3,69-71]. Adipose tissue is a highly active endocrine tissue that secretes numerous factors, including growth factors, cytokines, hormone-like molecules and many other molecules[72]. Several studies have demonstrated that the abundant number of growth factors such as vascular endothelial growth factor (VEGF), inflammatory cytokines [IL-6, tumor necrosis factor-alpha, interleukin-8 (IL-8)] and adipokines (adiponectin, leptin) released from adipose tissue can exert a substantial impact on the progression and outcome of many human diseases, including PC[3,16,71]. These molecules have a crucial role in obesity[73] and cell proliferation[74]. For instance, adipose tissue surrounding the prostate, i.e., periprostatic adipose tissue, is frequently invaded by prostate tumor cells, although its contribution to the tumor microenvironment is largely unknown. A recent study demonstrated that periprostatic adipose tissue produced local IL-6 at levels significantly higher than those in the circulation[67]. Other studies support a role for IL-6

production, which is up-regulated in obese patients, in PC cell migration and invasiveness[67,71].

Recently, Shariat *et al*[75] in a study of 423 preoperative and 206 postoperative blood samples from patients treated with radical prostatectomy for clinically localized PC investigated the association between sgp130 levels and PC prognosis. In the group of patients treated with radical prostatectomy, higher preoperative plasma sgp130 was significantly associated with higher pathological Gleason, extraprostatic extension, seminal vesicle invasion, lymph node metastasis and biochemical recurrence. These authors concluded that higher sgp130 plasma levels were associated with features of biologically aggressive PC. In a subset of 206 patients, postoperative sgp130 levels were 18% lower than preoperative levels. These decreased levels suggest that the higher blood levels of sgp130 are produced by tumor cells[75].

These clinical data support the biological role of the IL-6 pathway in PC, suggesting the inclusion of IL-6, sIL-6R and gp130 levels as new tumor biomarkers in PC patients[76]. Improving prediction accuracy by using more prognostic factors supports an early detection of any changes in the progression of the disease[25].

IL-6 expression in prostate cancer tissues and cell lines

The expression of IL-6 and its receptors has been investigated by several authors in benign prostate cells, PC tissues and in prostate cell lines (LNCaP, DU145, PC3). Hobisch *et al*[14] through immunohistochemical studies revealed that IL-6 expression was localized predominantly in basal cells of benign prostatic epithelium and in glandular cells of PC tissues. Another study observed that in normal prostate, IL-6 was immunolocalized in basal cells of the epithelium and gp130 was detected only in stromal cells[77]. However, Hobish *et al*[14] showed that cultured stromal cells secreted IL-6, but the rate of IL-6 was so low that there was only a minimal amount contained in the cells, and thus it may not be detectable by immunohistochemical methods. In benign prostate cells, gp130 was confined to the epithelium and stroma, and IL-6 was immunolocalized preferentially in epithelium[77]. These data are in agreement with Degeorges *et al*[78] who demonstrates that IL-6 is secreted by cultured benign prostate cells. In PC tissues, gp130 was detected in stroma and epithelium and the expression increased with Gleason Grade[77]. On the other hand, IL-6 was localized in all cell types and immunostaining increased with Gleason Grade[77]. These results are in agreement with the reported secretion of IL-6 by PC cells[79] and with increased IL-6 levels in PC patients with poor prognosis[42,48]. Palmer *et al*[80] observed that IL-6 and IL-6 receptors are expressed in PC cells. They showed that in LNCaP, DU145 and PC3 cell lines, this cytokine and its receptors are widely expressed, but not in normal prostate epithelial PZ-HPV-7 cells.

The role of the pro-inflammatory cytokine, IL-6, in PC lesions has not yet been clarified but may represent an interesting area of investigation. In order to delineate their specific functions during prostate tumor development and progression, several authors used human PC cell lines (LNCaP, DU145 and PC3)[17,81-88].

Significance of IL-6 pathway in cell growth: In vitro results

The castration-sensitive cell line, LNCaP, is one the most frequently used in PC studies. In order to improve our understanding of cellular events which may be relevant to PC patients with higher IL-6 levels, several authors treated LNCaP cells with IL-6[81,82].

Okamoto *et al*[82] found that growth of the LNCaP cell line was stimulated by the administration of IL-6, but not by their conditioned medium. Conditioned medium containing biologically active components (e.g., growth factors, cytokines) was obtained from previously cultured cells or tissues released into the media, substances that affect certain cell functions. These authors observed that DU145 and PC3 (castration-resistant) cell lines proliferate in response to stimulation with IL-6 and in response to its conditioned medium. The authors concluded that IL-6 acts as an autocrine and/or paracrine proliferative factor in PC cell lines[82]. It was demonstrated that IL-6 acts as a paracrine inducer of growth in the LNCaP cell line[17,79,82,83]. On the other hand, IL-6 functions as an autocrine growth-inducer in the DU145 and PC3 cell lines[82,84]. Chung *et al*[85] obtained similar results, finding that IL-6 acts as a growth inducer in PC3 and DU145 cell lines through an autocrine and paracrine action. However, the effect of IL-6 in LNCaP cells is still controversial. Some studies reported that IL-6 acts as an inducer of growth in this cell line[82,83,86], while others showed that IL-6 acts as an inhibitor[81,85,87]. The reason for these differences between the various studies is that IL-6 may have different functions in human PC cell line proliferation, according to their phenotypic characteristics.

Hobisch *et al*[81] generated a cell line (LNCaP-IL-6+) by exposing these cells to continuous administration of IL-6 and observed changes in their responsiveness and signal transduction. Initially, growth of LNCaP was inhibited by IL-6. After long-term treatment, the LNCaP-IL-6+ cell line began to secrete IL-6 and a higher basal proliferation rate was observed. In this situation, IL-6 induces cell growth.

Recently, there is evidence to suggest that IL-6 switches from a paracrine growth inhibitor to an autocrine growth stimulator[12,81,88]. Similar changes in responsiveness to IL-6 were observed in melanoma cells[89]. The behavior of the human prostate carcinoma LNCaP cell line may be dependent on the microenvironment of the culture system[12].

A recent study by Shariat *et al*[75] showed that sgp130 promotes PC invasiveness *in vitro*. In this study, continuous exposure of PC cells to sgp130 led to an increase in their invasiveness[75]. These authors suggest more studies are required with regard to the role of IL-6 and sgp130 in the PC biological behavior *in vitro* and *in vivo* for a better understanding of their role in this disease[75].

HORMONAL CASTRATION IN ADVANCED PROSTATE CANCER PATIENTS: ANDROGEN RECEPTOR AND IL-6 PATHWAYS

Initial treatment for organ-confined PC is usually radical prostatectomy and/or radiation therapy[90]. Although, most patients have advanced disease and are submitted to hormonal castration (HC)[90]. The initial response rate is excellent, but the majority of PC patients relapse into CRPC. CRPC is a common lethal form of PC that typically metastasizes to bone and visceral organs, frequently resulting in patient death[91,92]. Progression to metastatic disease is slow and can be accompanied by increased PSA levels[93]. The mechanisms implicated in CRPC progression are unknown. These findings suggest that CRPC progression remains the major obstacle to effective control and cure of advanced phenotype disease. Consequently, novel therapeutic strategies that target the molecular mechanism involved in CRPC are needed[94].

PC cells are androgen-dependent, in particular, testosterone is necessary for their growth and survival[91]. Thus, the blockade of testosterone initially causes a stop in PC cell growth[95]. However, not all tumor cells need testosterone for development. After HC, many tumors begin to exhibit a testosterone blockade resistance behavior. In this situation, CSPC cells undergo apoptosis and there is a selective advantage for CRPC cells with consequent proliferation[96].

The androgen receptor (AR) is an important protein involved in the normal maintenance, development and growth of prostate epithelial cells. AR is a nuclear ligand-activated transcription factor in the prostate gland and mediates the biological response of androgens with a crucial function in a molecular mechanism responsible for the transition from CSPC to CRPC progression[97-99]. The AR is expressed in the normal prostate and during various stages of prostate carcinogenesis (PIN, organ-confined tumors, metastatic tumors and before or after hormonal treatment)[100,101].

The AR uses di-hydrotestosterone (DHT) and testosterone as its natural ligands for phosphorylation, and the ligand-receptor complex translocates into the nucleus where it binds to a DNA sequence in the regulatory regions of AR target genes[99]. These complex interactions facilitate the activation or repression of the expression of several genes involved in the development, differentiation and proliferation of target cells. Some of these genes include *PSA* and *human glandular kallikrein 2* (*hk2*)[102].

During CRPC development, PC cells can develop alternative mechanisms which can influence their microenvironment and, consequently, their survival in an androgen-poor microenvironment[103]. Several models have been proposed to explain the development of this phenotype: hypersensitive pathways, promiscuous receptor, coactivators and corepressors, bypass pathway, PC stem cells and outlaw pathways[91,97,104-107].

In the hypersensitive pathways, the cells acquire the ability to use very low levels of androgen during HC[97]. There are three mechanisms that may or probably are involved in this pathway: (1) AR amplification, PC cells have increased expression of AR[103]. The increased AR expression allows higher ligand binding. This hypothesis is supported by results obtained with CRPC cell lines that show an increased expression of AR compared with CSCP[97]; (2) increased AR sensitivity, consequently, tumor cells are hypersensitive to the growth promoting effects of DHT[108]; and (3) increased 5-alpha-reductase activity that increases the conversion of testosterone to DHT[91].

The promiscuous pathway involves acquisition of mutations in the AR protein, consequently, the AR can be activated by nonandrogenic steroid molecules that are present in the circulation[91,97,105,106,109]. There are a large number of coactivators and corepressors involved in regulation of the AR[110]. These molecules are intermediates between the AR signaling pathway and transcriptional machinery[103]. In the bypass pathway, PC cells acquire a phenotype that allow them to survive and escape apoptosis in an androgen-depleted environment[106]. Another bypass mechanism is correlated with neuroendocrine differentiation of PC cells[106]. These cells are more represented in CRPC cells and are associated with a low rate of proliferation[106]. Neuroendocrine cells have the capacity to increase the proliferation of surrounding cells, thus progression of the PC cells occurs in a androgen-poor microenvironment[106].

The PC stem cells model showed that only a rare subset of cells is tumorigenic[111]. Collis *et al*[112] reported that a population of cells comprising 0.1% of prostate tumors (CD44+/a2h1/CD133+) without AR expression may be prostate cancer stem or progenitor cells. There is also another possible mechanism for cell survival in an androgen-poor microenvironment, where the presence of prostate cancer stem cells continually resupply the tumor cell population and are not affected by HC. These cells are capable of differentiating into androgen-dependent and -independent cells, leading to the development of a heterogeneous androgen receptor phenotype. This phenotype is typical in patients with CRPC[103,104].

Another potential hypothesis (outlaw pathway), is that during HC the AR can be activated by other nonsteroidal molecules synthesized and secreted by tumor cells, such as cytokines and growth factors [e.g., Keratinocyte Growth Factor, Epidermal Growth Factor, Insulin-like Growth Factor-1 and IL-6][113-115], leading to CRPC development and progression. Even if androgens are principally responsible for activating the AR, it is known that in the absence or presence of very low concentrations of androgens, the AR can be activated by growth factors and cytokines[113]. In prostate tumor, the microenvironment is secreting growth factors and cytokines which may directly manipulate paracrine and autocrine pathways involved in PC development and promote CRPC in patients treated with hormonal therapy[116].

Some studies have examined the IL-6 pathway in

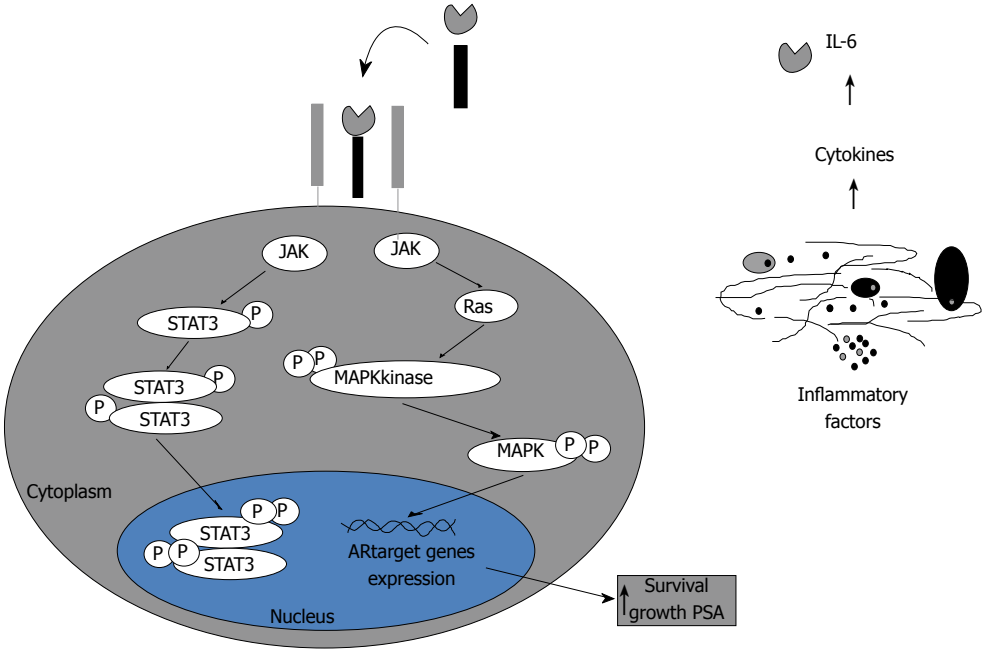

Figure 2 Hypothetical representation of AR pathway regulation by IL-6 in prostate cancer cells. IL-6: Interleukin 6; MAPK: Mitogen-activated protein kinase; STAT3: Signal transducer and activator of transcription 3; AR: Androgen receptor; PSA: Prostate-specific antigen.

CRPC progression and have revealed a possible involvement in regulation of the AR[117-119]. In the absence of androgen, IL-6 causes activation of the AR which is approximately 50% of the maximal activity induced by androgen. In low concentration conditions, androgen is potentiated by IL-6, leading to synergistic activation of the AR[93]. These observations demonstrate a cross-talk between the IL-6 pathway and AR. Due to the presence of increased IL-6 levels in patients with locally advanced or metastatic disease, the signaling pathway mediated by this cytokine could be an alternative pathway in the RCH phenotype acquisition and progression. Figure 2 shows that IL-6 activates the AR through a mechanism that is dependent on the MAPK and STAT3 signaling pathway in PC cells. This is a hypothetical representation of the AR pathway regulation by IL-6 in PC cells. The IL-6 pathway may be an alternative pathway for growth and proliferation of PC cells, under androgen deprivation conditions. IL-6 induces up-regulation of AR target gene expression such as PSA by STAT3 and MAPK signal transduction pathways. Figure 2 was adapted from[99,116].

We consider IL-6, other cytokines and growth factors as important regulators of PC growth with a significant role in the AR pathway. It has been proposed that the combined blockage of key molecules in proliferation signaling pathways (e.g., TGF, IL-6) could be one of the most promising strategies for CRPC[120]. Consequently, several authors have been investigating IL-6 activity in PC cells *in vitro* and *in vivo*. *In vitro* research on the regulation of PC cell growth and transcriptional activation of the AR by IL-6 have been focused on cell lines (LNCaP, DU145, PC3) and different results were obtained[17,82-84].

In vitro and in vivo studies on androgen receptor regulation by IL-6

The interaction between IL-6 and the AR may be more important in advanced PC patients who have elevated serum levels of IL-6 and its receptors. Several *in vitro* studies identified alternative pathways that influence or modify the activity of the AR signaling pathway in PC cells. Cross-talk between IL-6 and the AR was investigated in PC cells which transiently express the AR (DU-145) and in LNCaP cells which have a promiscuous mutated AR. Results on IL-6 induction, AR activation and tumor proliferation are contradictory. This is probably due to controversial results on the induction or stimulation effect of IL-6 on PC cell growth[121].

In LNCaP and DU-145 cell lines, IL-6 activates the AR in a ligand-independent and synergistic manner in low androgen concentrations[119]. This fact might seem paradoxical because proliferation of LNCaP was inhibited by IL-6. However, it has been suggested that IL-6 is a important molecule in the maintenance and differentiation of PC cells and this cytokine is involved in enhanced PSA mRNA regulation and in the regulation of its protein[93]. It was observed that long-term treatment with IL-6 in LNCaP cells increased expression and activity of the AR[117,119] and overexpression of IL-6 protects LNCaP cells from apoptosis induced by HC[93].

In MDA PC 2b cells (androgen-sensitive cell line), IL-6 promotes growth and this effect was dependent of the AR[76]. It is known that AR signaling is complex and there are several AR-associated proteins with a crucial role in the modulation of response in a cell type-dependent manner[122]. For this reason, IL-6 up-regulation has been investigated in PC. Additionally, authors hypoth-

esized that IL-6 may promote tumor growth through AR activation *in vivo*. In their work, MDA PC 2b cells were xenografted into nude mice. They observed that growth of MDA PC 2b xenografts in castrated animals treated with IL-6 was similar to that in non-castrated animals. In addition, tumors did not significantly grow in castrated mice and mice treated with IL-6 and bicalutamide (oral non-steroidal anti-androgen used in the treatment of PC). Bicalutamide showed an inhibitory effect on IL-6-regulated growth *in vivo*[76].

This evidence demonstrated that IL-6 has a crucial role in androgen-responsive gene expression and, consequently, is an important regulator in the growth of CRPC cells *in vitro* and *in vivo*. Another recent study evaluated the effects of IL-6 in the LNCaP cell line on phenotype changes before and after androgen deprivation[94]. *In vitro* observations indicate that the growth of LNCaP/IL-6 (IL-6-transfected LNCaP) was significantly lower than LNCaP/Co (not IL-6-transfected LNCaP) under androgen-deprivation conditions. Furthermore, LNCaP/IL-6 tumors in nude mice rapidly regressed after castration. However, LNCaP/Co tumor growth was transiently inhibited after castration and then continuously accelerated[94]. Gene microarray analyses showed that androgen deprivation resulted in the differential expression of genes involved in growth, apoptosis and carcinogenesis between LNCaP/Co and LNCaP/IL-6[94]. The principal conclusion of this study is that IL-6 produced by LNCaP may have a suppressive role on growth and a crucial function in the androgen-resistance phenotype under an androgen-poor microenvironment[94]. These and other future investigations will clarify the molecular mechanism involved in changes of phenotype in LNCaP cells that express higher IL-6 levels.

Efficient activation of the AR by IL-6 involves multiple signaling pathways such as STAT3, PI3K and MAPK. At present, little is known about these mechanisms. Some studies have used inhibitors of these signaling pathways. Inhibitors of the JAK, MAPK and PI3K signaling pathways resulted in a down-regulation of IL-6 on the AR[119]. Lin *et al*[117] showed that MAPK is important for AR activity through IL-6. These authors demonstrated that an inhibitor of MAPK abrogated IL-6 activation of the PSA promoter. In contrast, an inhibitor of the PI3K pathway had no effect on IL-6 regulation of AR activity[117]. Chen *et al*[118] also showed that JAK inhibition stopped AR activation by IL-6. This study demonstrated that the JAK-STAT pathway is implicated in AR activation by IL-6 in the LNCaP cell line. On the other hand, Zelivianski *et al*[123]. demonstrated that IL-6 up-regulated AR protein levels in low passage LNCaP cells, while in high passage LNCaP cells the opposite effect was observed.

Recent research in prostate carcinoma has focused on the role of sIL-6R. The trans-signaling pathway mediated by complex IL-6/sIL-6R seems to have an important role in the pathophysiology of certain inflammatory, nervous system and cardiovascular diseases and some cancer types[34]. The IL-6/sIL-6R complex can have an antiapoptotic role and is therefore considered a possible cause of certain cancers such as colon cancer and melanoma[26,34,52,124-127]. To the best of our knowledge, the classical and trans-signaling pathways in specific PC events are not well known. Furthermore, sIL-6R showed a stronger association with disease progression than IL-6[54], suggesting a role of complex IL-6/sIL-6R in the spread of metastases. Santer *et al*[54] conducted a study with the principal aim of determining the effect of complex IL-6/sIL-6R on PC cell proliferation and in metastasis formation. These authors focused their studies on sIL-6R, because it is the first element in the IL-6 signaling pathway. They found that activation of sIL-6R resulted in increased PC cell motility and migration[54]. Thereby, it is believed that sIL-6R may be important in the metastastic process through down-regulation of the tumor suppressor, maspin, by sIL-6R. In contrast, the IL-6 trans-signaling pathway reduces PC cell proliferation[54].

It was suggested that targeting sIL-6R may be an alternative method of improving anti-IL-6 therapies used in PC treatment[54]. Understanding how the IL-6 pathway affects cellular events in the PC cell microenvironment and its interaction with the AR pathway will allow the development of preventive and therapeutic strategies for PC patients in the future.

IL-6 TARGETING

The importance of the IL-6/IL-6R pathway in the regulation of PC cells and the potential involvement of this signaling pathway in androgen-resistant growth of PC cells makes it a good candidate for targeted therapy. In this perspective, the use of IL-6-neutralization antibodies, antisense oligonucleotides and antagonists should be the subject of study[88]. The principal goal is to identify new therapies to target tumor cells and/or microenvironment and consequently increase the chances of survival for PC patients with aggressive phenotypes and those who develop resistance to hormonal therapy.

It has been reported that IL-6R blockade by IL-6R antagonists might reduce tumor cell growth and consequently disease progression[128]. The IL-6/IL-6R signaling pathway involves numerous proteins and a large number of phosphorylation cascade pathways. Downstream molecules of these proteins and pathways may be crucial targets for specific therapies. For example, inhibition of STAT3 suppresses PC progression[129] and reduces STAT3 target gene expression, such as VEGF, Bcl-X and cyclin D1 and leads to apoptosis[130].

Other investigations are involved in the study of the chimeric monoclonal anti-IL-6 antibody, siltuximab (CNT0 328). Steiner *et al*[131] showed that tumor growth in nude mice inoculated with LNCaP-IL-6+ cells after CNTO 328 treatment was reduced. Other studies obtained analogous results when PC3 and LuCaP 35 xenografts were treated with CNTO 328[132,133]. Another study reported that CNT0 328 can inhibit PC cell growth *in vitro* and improve survival by reducing the level of ca-

Table 1 Summary of several studies on IL-6, sIL-6R and sgp130 levels in prostate cancer patients and targeted therapies for the IL-6 signaling pathway

	Ref.	Conclusions
Prognostic implications	[15,16]	L-6 level is a significant prognostic factor for PC. A significantly shorter survival in PC patients was associated with elevated IL-6 levels, serum PSA levels and aggressive disease
	[48,57-60]	The serum levels of IL-6 were significantly higher in PC patients with metastatic disease
	[48,62-66]	The levels of IL-6, sIL-6R and TGF-β1 predicted biochemical recurrence after surgery or radical prostatectomy
	[75]	In patients treated with radical prostatectomy higher preoperative plasma sgp130 was significantly associated with higher pathological Gleason, extraprostatic extension, seminal vesicle invasion, lymph node metastasis and biochemical recurrence. The postoperative sgp130 levels were 18% lower than preoperative levels
Therapeutic implications	[131-133]	These studies involved the chimeric monoclonal anti-IL-6 antibody, siltuximab (CNT0 328) It was shown that tumor growth in nude mice inoculated with LNCaP-IL-6+ cells after CNTO 328 treatment was reduced. Analogous results were obtained when PC3 and LuCaP 35 xenografts were treated with CNTO 328
	[135]	The administration of siltuximab in a group of patients who had already received docetaxel therapy had no clinical efficacy
	[136]	PC cells can develop resistance to docetaxel and STAT1 is increasingly expressed in docetaxel-resistant PC cells
	[84]	The treatment of the PC3 cell line with Sant7 inhibits cell growth more efficiently than other anti-IL-6 antibodies
	[134]	CNT0 328 can inhibit PC cell growth *in vitro* and improve survival by reducing the level of cachexia in an animal model of PC
	[133]	In mice, CNT0 328 inhibited the conversion of CSPC into more aggressive disease
	[137]	STAT3 and MAPK activity is suppressed in patients taking siltuximab, which may inhibit IL-6-mediated drug resistance

PC: Prostate cancer; CSPC: Castration-sensitive prostate cancer; TGF-β1: Transforming growth factor-beta1; IL-6: Interleukin-6; sIL-6R: Soluble interleukin-6 receptor; STAT3: Signal transducer and activator of transcription 3; MAPK: Mitogen-activated protein kinase.

chexia in an animal model of PC[134]. In addition, CNT0 328 has been shown in mice to inhibit the conversion of CSPC into more aggressive disease, bone metastasis, and difficult to treat CRCP[133].

In a clinical trial, it was shown that the administration of siltuximab in a group of patients who had already received docetaxel therapy had biological, but not clinical efficacy[135]. PC cells can develop resistance to docetaxel, and Patterson *et al*[136] reported that STAT1 is increasingly expressed in docetaxel-resistant PC cells. The high heterogeneity of prostate tumors can explain this resistance to docetaxel in PC cells[11]. Recent studies indicate that STAT3 and MAPK activity is suppressed in patients taking siltuximab, which may inhibit IL-6-mediated drug resistance[137]. However, in a study that involved patients with CRPC, where the disease had progressed beyond docetaxel therapy, siltuximab had a minimal clinical effect, despite positive biological IL-6 inhibition[135].

Lou *et al*[83] reported that targeting IL-6 may have multiple advantages in patients that receive limited therapeutic and survival benefit from conventional therapies. In a previous study, Borsellino *et al*[84] found that treatment of the PC3 cell line with Sant7, a modified interleukin-6 which binds with high affinity to IL-6R but does not bind with gp130, inhibits cell growth more efficiently than other anti-IL-6 antibodies.

We believe that the development and availability of IL-6 inhibitors is fundamental for the treatment of IL-6-dependent cancers, where the IL-6 signaling pathway is deregulated. This review demonstrates the role of IL-6 and the levels of its receptors as prognostic factors in PC patients. These deregulated levels could be important in anti-IL-6 therapy development. Table 1 presents a summary of several studies on IL-6, sIL-6R and sgp130 levels in PC patients and targeted therapies for the IL-6 signaling pathway. However, more studies and appropriate clinical trials are needed to determine the effectiveness of anti-IL-6 therapies in cancer patients.

CONCLUSION

The information presented in this review suggests that the IL-6 signaling pathway plays an important role in PC development/progression, and IL-6 is able to maintain tumor growth through the AR pathway in androgen-deprived conditions. Further studies are suggested to assess the functionality of the IL-6/sIL-6R complex in PC. Understanding how IL-6 affects cellular events in the PC cell microenvironment and its interaction with the AR pathway will allow the development of preventive and therapeutic strategies for PC patients in the future. However, it is also important to study and characterize other signaling pathways involved in CRPC progression and the cross-talk among them, allowing the design of new and more adequate targeted therapies. Additionally, diverse studies reported that serum and plasma levels of IL-6 and sIL-6R are increased in patients with aggressive disease and a poor prognosis, suggesting the inclusion of IL-6 and the levels of its receptors as putative new tumor biomarkers. In addition, changes in serum IL-6 levels could help direct additional treatment strategies in the future, however, clinical studies are needed to assess this potential.

In conclusion, IL-6 is a good candidate for the development of targeted therapies in PC, but more studies and appropriate clinical trials need to be carried out to ascertain the effectiveness of anti-IL-6 therapies in PC patients.

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BRIEF ARTICLE

Clinical utility of cerebrovascular reactivity mapping in patients with low grade gliomas

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Abstract

AIM: To evaluate neurovascular uncoupling (NVU) associated with low grade gliomas (LGG) using blood oxygen level dependent (BOLD) cerebrovascular reactivity mapping.

METHODS: Seven patients with low grade gliomas referred by neurosurgeons for presurgical mapping were included in this pilot study. Cerebrovascular reactivity (CVR) mapping was performed by acquiring BOLD images while patients performed a block-design breath-hold (BH) hypercapnia task. CVR mapping was expressed as BOLD percentage signal change (PSC) from baseline associated with performance of the BH hypercapnia task. Standard T2* Dynamic Susceptibility Contrast perfusion imaging was performed and relative cerebral blood volume (rCBV) and relative cerebral blood flow (rCBF) maps were generated. Structural T1 weighted MR images were also acquired. A correlation analysis between intratumoral normalized (*via* ratio with contralateral homologous regions) BOLD BH PSC [referred to as (n_{CVR})] and intratumoral normalized resting state rCBV (rCBF) values (i.e., n_{CBV} and n_{CBF}, respectively) was performed.

RESULTS: No significant correlation was seen between the normalized BOLD BH PSC (i.e., n_{CBV}) and n_{CBV} or n_{CBF}. However, the average n_{CVR} (median = 0.50, z = -2.28, P = 0.01) was significantly less than 1.0, indicating abnormally reduced vascular responses in the tumor regions relative to normal contralesional homologous regions, whereas the average n_{CBV} (median = 0.94, z = -0.92, P = 0.375) and n_{CBF} (median = 0.93, z = -1.16, P = 0.25) were not significantly higher or lower than 1.0, indicating iso-perfusion in the tumor regions relative to normal contralesional homologous regions. These findings suggest that in LGG, hyperperfusion that is seen in high grade gliomas is not present, but, nevertheless, abnormally decreased regional CVR is present within and adjacent to LGG. Since the patients all demonstrated at least some residual function attributable to the cortical regions of impaired CVR, but were incapable of producing a BOLD response in these regions regardless of the tasks performed, such regionally decreased CVR is indicative of NVU. The low n_{CVR} ratios indicate high prevalence of NVU in this LGG cohort, which is an important consideration in the interpretation of clinical presurgical mapping with functional magnetic resonance (MR) imaging.

CONCLUSION: Our preliminary study shows that BH CVR mapping is clinically feasible and demonstrates an unexpectedly high prevalence of NVU in patients with LGG.

Key words: Blood oxygen level dependent; Brain tumor; Cerebrovascular reactivity; Functional MRI; Neurovascular uncoupling; Presurgical mapping

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INTRODUCTION

Presurgical localization of brain sensorimotor, visual and language regions in patients with brain tumors or epilepsy who are candidates for surgical resection currently represents the most mature clinical application of blood oxygen level dependent (BOLD) functional MR imaging (fMRI)[1-3]. Presurgical mapping with fMRI can assist neurosurgeons by providing useful information for: (1) preoperative risk assessment; (2) planning the safest surgical trajectory; (3) selection of patients for asleep *vs* awake craniotomy; and most importantly; and (4) optimization of efficiency, exposure and technique of intraoperative mapping. However, despite its many advantages in presurgical planning that have resulted in widespread clinical utilization over the last decade, some limitations of clinical fMRI do exist[4-9]. One such limitation is the frequent inability to distinguish essential from nonessential participatory activated (i.e., "eloquent") cortex involved in performance of a particular cognitive, sensorimotor or visual task, thus leading to lower than ideal specificity of activation maps[10].

Another limitation is the problem of decreased sensitivity for detection of actual electrically active eloquent cortex in areas of impaired cerebrovascular reactivity (CVR); this phenomenon is referred to as neurovascular uncoupling (NVU)[11,12]. NVU can result in false negative BOLD activation, which constitutes a major hazard with respect to interpretation of clinical fMRI examinations; such false negatives within or adjacent to tumor boundaries can result in undesirable resection (in the absence of intraoperative electrophysiologic confirmation) of essential electrically active eloquent cortex that is incapable of demonstrating a BOLD response due to impaired CVR. Such eloquent cortical resection may lead to serious permanent postoperative neurological deficits. Thus, the phenomenon of NVU is not merely a theoretical issue of scientific interest, but is rather an issue of considerable clinical relevance and importance. NVU has been documented in the immediate vicinity of high grade gliomas (HGG), mostly due to tumor angiogenesis, which is associated with abnormal vascular permeability, abnormal hyperperfusion (elevated relative cerebral blood volume [rCBV]) related to increased vascular density, and impaired regional CVR[13,14]. However, it is not clear how high the prevalence of impaired CVR (and resultant NVU) is in low grade gliomas (LGG), in which hyperperfusion is unusual. In this study, we investigated regional CVR, using a BOLD breath-hold (BH) hypercapnia task, within LGG, which are known to infiltrate, rather than destroy or displace, eloquent cortex, in order to determine whether the same NVU potential exists in these tumors as in HGG. In this study we report our initial experience using BH CVR mapping in 7 patients with LGG (6 patients with grade II gliomas and 1 patient with grade I glioma) as a quality control tool for detecting risk of NVU, and we compare these results to those of T2* DSC perfusion imaging that was also performed during the same scan sessions as part of a comprehensive clinical presurgical mapping protocol. The findings of this study are discussed in the context of current literature pertaining to brain tumor-related NVU.

Table 1 Age, sex, tumor location and histology for seven brain tumor cases included in the study

Age	Sex	Tumor location	Histology/tumor grade
25	F	Left frontal lobe	Oligoastrocytoma grade Ⅱ
27	M	Right cingulate gyrus	Oligodendroglioma grade Ⅱ
42	M	Left temporal lobe	Astrocytoma grade Ⅱ
25	M	Right temporal lobe	Diffuse astrocytoma grade Ⅱ
54	M	Left frontal	Oligodendroglioma grade Ⅱ
27	F	Left hemispheric (primarily insular and inferior frontal)	Pilocytic astrocytoma grade Ⅰ
41	M	Left insular	Oligoastrocytoma grade Ⅱ

F: Female; M: Male.

MATERIALS AND METHODS

Seven patients (mean age 34 ± 11 year, 5M/2F) with histopathologically proved grade Ⅰ and Ⅱ intra-axial primary brain tumors (Table 1) underwent our institutional clinical BOLD fMRI protocol for presurgical planning which included multiple T2* BOLD fMRI sequences during performance of motor, language or visual tasks and a BH task, a T2* Dynamic Susceptibility Contrast perfusion sequence and a structural T1-weighted 3D MPRAGE sequence after Gadolinium injection. Details of these three sequences are reported in Table 2. Images were acquired on a 3T MRI scanner (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany). The block design BH task consisted of four cycles of 40 s each of normal breathing (baseline) alternating with blocks of 4 s of inhalation and 16 s of breath-holding[15]. Instructions for task performance were delivered visually using Prism Acquire Software (Prism Clinical Imaging, Elm Grove, WI, United States).

The study was approved by our Institutional Review Board. Images for each patient were first transferred to an external workstation. Perfusion and raw BOLD BH images were coregistered to the T1 MPRAGE images using DynaSuiteNeuro software (DynaSuiteNeuro, InVivo Corporation, Pewaukee, WI, United States). Perfusion image analysis included the generation of rCBV and rCBF maps. rCBV was calculated by adding a correction

Table 2 Main parameters for the sequences of interest in the study

Sequence	TR (ms)	TE (ms)	FA	FOV (cm^2)	Acquisition matrix	Slice thickness (mm)
T1 MPRAGE	7	3.5	9°	24 × 24	256 × 256	1
T2* DSC	2450	45	90°	24 × 24	128 × 128	4
T2* BOLD	2000	30	90°	24 × 24	64 × 64	4

DSC: Dynamic susceptibility contrast; BOLD: blood oxygen level dependent; FOV: Field of view.

factor to take into account the contrast leakage through the disrupted blood-brain barrier[16]. BOLD BH data analysis was carried out using AFNI software (afni.nimh.nih.gov) and included slice timing correction, realignment, spatial smoothing followed by generation of PSC maps[17]. Subsequently, a Region of Interest (ROI) analysis was carried out using MIPAV (mipav.cit.nih.gov) (Medical Image Processing, Analysis and Visualization) software. For each patient, two independent raters [JJP (a board-certified neuroradiologist with 14 years of neuroimaging experience) and DZ (an imaging scientist with a PhD in functional imaging and 3 years of postdoctoral neuroimaging experience)] selected on the high resolution T1 MPRAGE images a ROI that included the tumor entirety, defined as the entire hypointense component. This ROI encompassing the entire lesion shall be referred to as the "ipsilesional ROI." This T1 hypointense region corresponded exactly to the areas of tumor T2/FLAIR hyperintensity seen on other sequences acquired as part of the overall clinical fMRI examination, but T1 MPRAGE images were selected for ROI delineation because of their higher resolution compared to the standard FSE T2 and T2 FLAIR sequences. A mirror homologous contralateral hemispheric (referred to as "contralesional") ROI was generated in a semi-automated fashion, with particular attention paid to trying (to the greatest extent possible) to ensure a similar degree of contribution from gray and white matter structures in the contralesional ROI as in the ipsilesional ROI, considering the degree of anatomic distortion resulting from the tumor. The following metrics were then calculated: a normalized rCBV ($nCBV$), expressed as the ratio between the mean rCBV value (of all included voxels) in the ipsilesional ROI and the mean rCBV value in the contralesional ROI; a similarly computed normalized rCBF ($nCBF$), defined as the ratio between the mean rCBF value in the ipsilesional ROI and the mean rCBF value in the contralesional ROI; a normalized PSC ($nCVR$) expressed as the ratio between the mean BOLD PSC in the ipsilesional ROI and the mean BOLD PSC in the contralesional ROI.

Correlation analysis was performed between $nCBV$ and $nCVR$ as well as between $nCBF$ and $nCVR$. Mean values among the raters were used. A one sample Wilcoxon test was also performed to assess whether the PSC normalized ratio was significantly lower than 1.0. An identical statistical test was also perfomed on $nCBV$ and $nCBF$ to determine whether there were any significant differences in perfusion metrics between the ipsilesional and contralesional ROIs. Statistical analysis was performed using OriginPro 8.0 software.

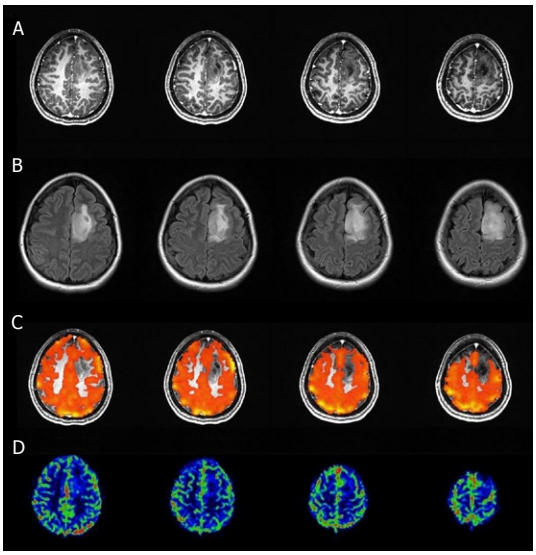

Figure 1 Axial T1 postcontrast 3D MPRAGE and T2 FLAIR images (A and B) breath-hold cerebrovascular reactivity maps (C) and relative cerebral blood flow maps (D) in a patient presenting with a left frontal lobe lesion, classified as grade II oligoastrocytoma after surgical resection, are displayed. Breath-hold cerebrovascular reactivity (BH CVR) maps were fused with axial T1 post Gadolinium images, and the threshold was set to 0.35 for blood oxygen level dependent PSC. Decreased CVR is present within and at the anterosuperolateral margin of the lesion (involving the infiltrated cortex of the left superior frontal gyrus) relative to contralateral hemispheric normal tissue. This represents an area of tumor-induced neurovascular uncoupling. Perfusion imaging did not provide equivalent information because the same area does not demonstrate any definite regional perfusion abnormality.

RESULTS

T1 MPRAGE and T2 FLAIR images, perfusion maps and BH PSC maps for two cases are shown in Figures 1 and 2. Reduced PSC is clearly visible in the ipsilesional ROI compared to the contralesional ROI, whereas in both cases the lesion appears iso-perfused relative to the contralesional ROIs. Intraclass Correlation Coefficients (ICC) among the raters were excellent (0.88 for $nCVR$, 0.98 for $nCBV$, 0.98 for n $nCBF$). In this group of 7 patients, none of the cases demonstrated a $nCVR$ that was greater than 1.0 (Figure 3A). The fact that all 7 cases demonstrated $nCVR$ values less than 1.0 indicated that every one of the LGG cases demonstrated abnormally decreased CVR in the ipsilesional ROI compared to the contralesional ROI, suggesting a high risk of NVU in all cases in areas of cortex where regionally decreased CVR were noted. Correlation with the patients' clinical status confirmed that these areas of regionally decreased cortical CVR corresponded to areas of actual NVU, since preservation of residual motor and language function was noted clinically despite absent expected task-based activation in these regions of decreased CVR on task-based fMRI activation maps obtained as part of the concurrent clinical fMRI examinations. Based on group

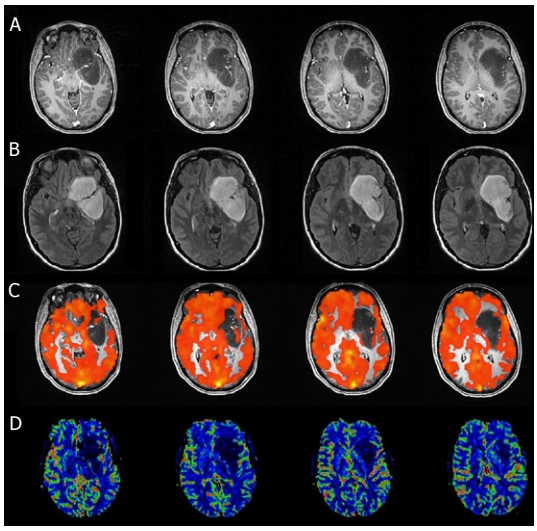

Figure 2 Axial T1 postcontrast 3D MPRAGE and T2 FLAIR images (A and B), breath-hold cerebrovascular reactivity maps (C) and relative cerebral blood flow maps (D) in a patient presenting with a left hemispheric lesion, classified as pilocytic grade I astrocytoma after surgical resection, are displayed. Breath-hold cerebrovascular reactivity (BH CVR) maps were fused with axial T1 post Gadolinium images, and the threshold was set to 0.45 for blood oxygen level dependent PSC. Decreased CVR is present within the lateral margin of the lesion (involving the adjacent cortex of the left superior temporal gyrus) relative to contralateral hemispheric normal tissue. This represents an area of tumor-induced neurovascular uncoupling. Perfusion imaging did not provide equivalent information because the same area does not demonstrate regional perfusion abnormality.

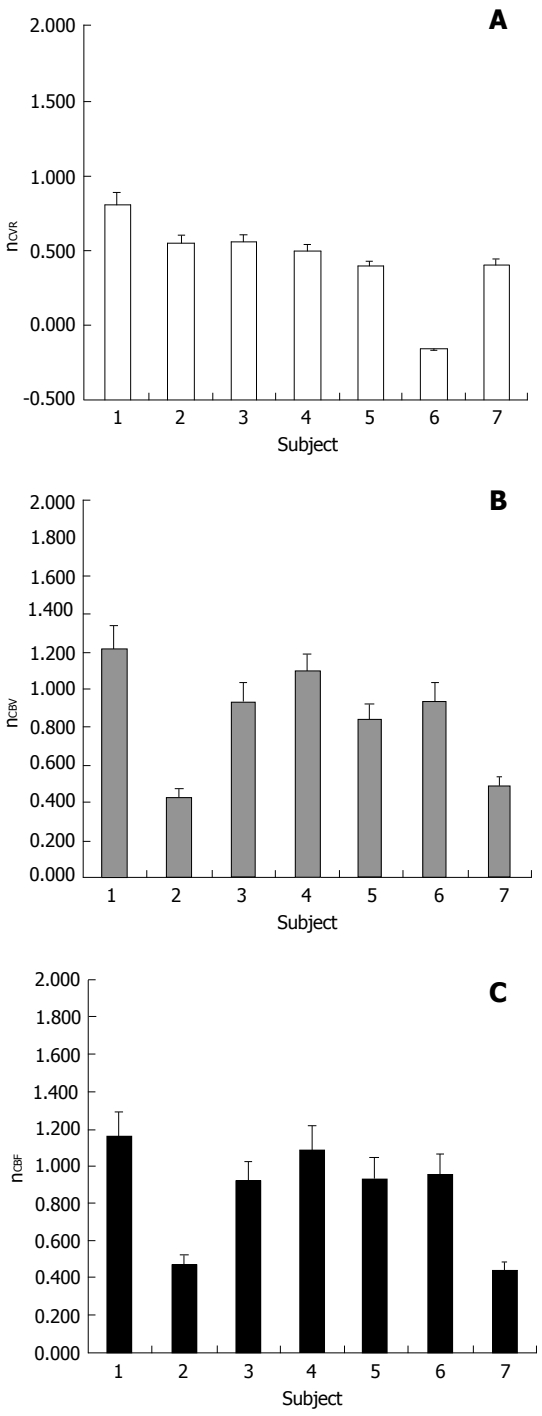

Figure 3 Cerebrovascular reactivity ratio (n_{CVR}) (A) cerebral blood volume ratio (n_{CBV}) (B) and cerebral blood flow ratio (n_{CBF}) (C) distribution for the group of seven patients with classified grade I and grade II tumors included in this study. In all cases n_{CVR} is less than 1.0 and the median value is significantly less than 1.0 according to the one sample Wilcoxon test. n_{CBV} and n_{CBF} median values are not significantly lower or higher than 1.0.

analysis, the overall distribution of n_{CVR} in this cohort of patients was statistically significantly lower than 1.0 (median = 0.50, z = -2.28, P = 0.01). n_{CBV} (median = 0.94, z = -0.92, P = 0.375) and n_{CBF} (median = 0.93, z = -1.16, P = 0.25) were not significantly higher or lower than 1.0 at a group level, indicating the absence of any substantial hyperperfusion or hypoperfusion in the ipsilesional ROI compared to the contralesional ROI (Figure 3B and C). We did not find any significant correlation between the perfusion and CVR metrics (Figure 4A and B), indicating that perfusion imaging by itself is not a valid predictor of vascular reactivity, and therefore an indicator of NVU in this particular cohort of LGG patients.

The use of a semi-automated approach to contralesional ROI placement helped to ensure that similar contributions to the contralesional ROI from gray and white matter structures were obtained as in the ipsilesional ROIs. This avoided spuriously high contralateral perfusion and CVR values related to greater contributions from normal gray matter in the contralesional ROIs. This was especially important considering that all of the lesions were gliomas, and as such involved mostly white matter rather than cortical gray matter. None of the 7 LGGs demonstrated any appreciable contrast enhancement on Gadolinium-enhanced T1 weighted images, and none demonstrated any internal areas of necrosis by histopathology or imaging features, although some very small regions of internal cystic change were noted in some of the oligodendrogliomas in this group. It is important to note that no internal necrosis or enhancement was present, since such features may result in spuriously decreased or increased mean perfusion, respectively, in ipsilesional ROIs. In the patients with oligodendrogliomas in our cohort, the known propensity toward relatively higher tumor perfusion than comparable low grade astrocytomas is balanced by propensity for internal cystic

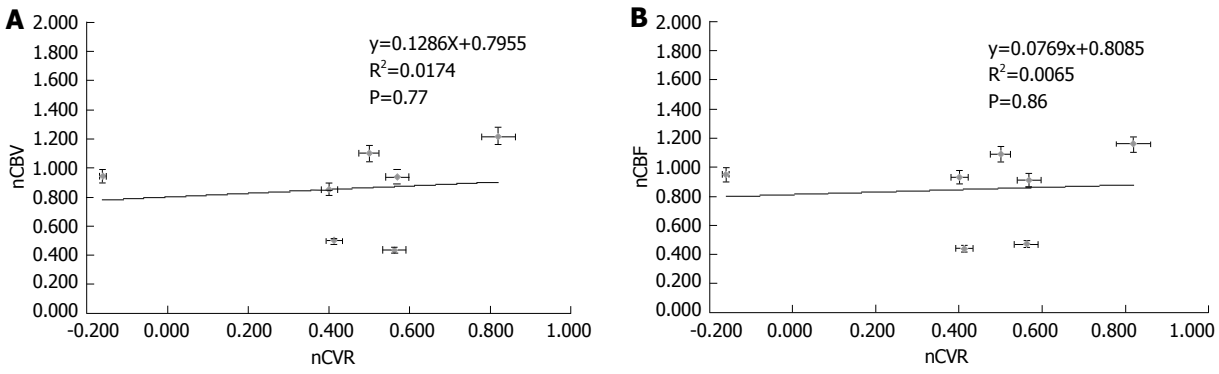

Figure 4 Normalized relative cerebral blood volume ratio (n_{CBV}) *vs* normalized cerebrovascular reactivity ratio (n_{CVR}) (A) and normalized relative cerebral blood flow ratio (n_{CBF}) *vs* normalized CVR ratio (n_{CVR}) (B) in our cohort of low grade gliomas. As described in the Methods section, these normalized ratios were calculated by dividing the mean voxel values obtained in ipsilesional regions of interest (ROIs) by those obtained in mirror contralesional ROIs. Neither n_{CBF} nor n_{CBV} demonstrated significant inverse correlation with n_{CVR} in this LGG group. LGG: Low grade gliomas; CVR: Cerebrovascular reactivity

change, thus not resulting in overall mean voxel hyperperfusion within the ipsilesional ROIs. By using overall mean perfusion metrics computed from all voxels within the ROI rather than simply voxels with maximal perfusion metrics within the ROI, we avoided the risk of spuriously high perfusion values contributing to artifactually high perfusion ratios.

DISCUSSION

All of the LGG cases in our study demonstrated reduced CVR in the tumor (i.e., ipsilesional) ROI compared to the normal contralateral hemispheric mirror (i.e., contralesional) ROI. Such regionally abnormally reduced CVR, despite the presence of clinically intact, albeit impaired, motor or language function in all of these patients is direct evidence of tumor-related NVU. The fact that no substantial corresponding regional perfusion abnormality was present in any of these cases is reflected in the absence of significant correlation between the normalized (i.e., ipsilesional to contralesional) perfusion ratios and normalized CVR ratios. The absence of tumor hyperperfusion is expected in this cohort of LGG, since such tumors, unlike HGG, are not associated with angiogenesis[18,19]. Although reports of NVU associated with hyperperfusion in HGG exist in the literature[20,21], few reports of NVU related to LGG exist[22,23]. We have shown in our study that the phenomenon of NVU, as detected by regionally decreased CVR, in LGG is much more prevalent than previously thought[24]. We have also shown the clinical feasibility of the performance of such BH CVR mapping in such a patient population. Our results suggest that BH CVR mapping should be considered in all brain tumor patients regardless of tumor grade.

The coupling mechanism between neuronal firing and blood flow changes results from a complex sequence (which we can consider as the NVU cascade) of cellular, metabolic and vascular processes involving neurons, glial/astrocytic components, neurotransmitters, chemical mediators and eventually vascular smooth muscle cells. The currently accepted explanation is that during neuronal activity, synaptic release of neurotransmitters, such as glutamate, that bind to receptors on other neurons, may trigger the secondary release of vasodilatory mediators such as nitric oxide, which in turn increase CBF and CBV. These neurotransmitters such as glutamate can also act on astrocytes through different receptors, thus resulting in the release of compounds such as arachidonic acid and prostaglandin E2, which in turn result in vasoconstriction or vasodilatation, respectively, by acting on arteriolar smooth muscle[25]. It is possible that while in HGG, aberrant neovascularity with abnormal permeability and vasoactivity may be primarily responsible for the NVU, in LGG, abnormal astrocytic function or dysfunction involving other elements of the NVU cascade may be responsible. However, little is known about the pathophysiologic mechanisms underlying such NVU in LGG.

The need to detect NVU, when present, is critical in the interpretation of clinical fMRI examinations because regional cortical NVU will result in an inability to elicit BOLD activation in the affected cortex regardless of the nature of the particular fMRI task performed. Thus, false negative activation in these cortical regions may result during performance of sensorimotor, visual, language or other cognitive tasks that are expected to activate such regions based on a priori knowledge of functional anatomy. Such false negatives may not only result in incorrect language lateralization, but also incorrect localization or underestimation of the true extent of localization of eloquent cortex, as well as possibly incorrect inferences regarding tumor-induced cortical functional reorganization[11]. Such erroneous interpretations of task-related fMRI activation maps can result in unexpected and tragic postoperative neurological deficits related to inadvertent resection of eloquent cortex that is "BOLD-silent" directly due to NVU. The added value of BH CVR mapping in this setting lies in the additional confidence in assignment of function to areas of activation on fMRI maps in cases where no regional CVR abnormality results, as well as in proper exercising of caution in cases where functional activation is expected on a particular task in a particular cortical region where absent activation

is seen on task-based fMRI with corresponding abnormally decreased regional CVR. In the latter case, such as in this LGG cohort, one needs to acknowledge the limitation of clinical fMRI and needs to inform the referring neurosurgeon that complementary intraoperative electrophysiologic mapping will be necessary to exclude eloquent cortex in these regions of impaired CVR adjacent to or within the LGG. Such knowledge is very useful, in our clinical experience, in neurosurgical planning as well as in counseling of patients regarding the potential risks of tumor resection.

Thus, in conclusion, although our results are preliminary and based on a fairly small sample size, they suggest that BH CVR mapping in patients with LGG is both clinically feasible and capable of detecting NVU, which is a critical limitation of clinical fMRI. We furthermore note an unexpectedly high prevalence of NVU in LGG, suggesting that NVU is a commonly encountered phenomenon in brain tumors of all grades, and not just in HGG as previously thought.

COMMENTS

Background

Cerebrovascular reactivity (CVR) mapping using a breath-hold (BH) technique is a method of evaluating how responsive the microvasculature in the brain is to external stimuli. Although the mechanism for BH CVR mapping is related to transient mild increases in pCO2 (i.e., hypercapnia), resulting in vasodilatation, this can be applied to the evaluation of standard clinical blood oxygen level dependent (BOLD) functional MRI (fMRI) examinations, where sensorimotor, visual or language/cognitive stimuli result in transient blood flow changes in brain microvasculature adjacent to activated neurons. If CVR is impaired for any reason, such as aberrant tumor neovascularity or astrocytic dysfunction due to tumor infiltration, then no BOLD response is possible on standard clinical fMRI activation studies because the BOLD response relies on intact CVR.

Research frontiers

It has been determined that CVR is impaired within or adjacent to low grade gliomas (LGG), thus compromising our ability to accurately map eloquent cortex for surgical planning using fMRI. It has already been established that such impaired CVR is present in high grade gliomas (HGG) due to tumor angiogenesis as reflected in hyperperfusion on MR perfusion imaging.

Innovations and breakthroughs

Very little is currently known about CVR in LGG. Most of the work to date relating to CVR mapping in brain tumor patients relates to applications in HGG. It is clear that tumor hyperperfusion, as detected on T2* DSC (standard clinical) MR perfusion imaging, is related to tumor angiogenesis, but in LGG, angiogenesis typically does not occur. Astrocytic dysfunction, however, is known to occur due to tumor infiltration and primary astrocytic and/or oligodendrocyte involvement by all LGGs.

Applications

The findings of a high prevalence (100% in our cohort) of abnormal CVR in LGG is of immense clinical value, because this is a potentially serious limitation of standard clinical BOLD fMRI examinations that may result in false negatives which adversely impact surgical planning. The recognition of this pitfall of fMRI is critical for proper surgical planning and counseling of patients prior to surgical resection of LGG. Further studies with larger sample sizes will need to be performed to evaluate the true prevalence of such findings in LGG of different histologic subtypes.

Terminology

Cerebrovascular reactivity mapping and BOLD fMRI have been described in the Background section above. BOLD fMRI is a method of noninvasively evaluating sensorimotor, visual, language and other cognitive functions and mapping eloquent cortical regions prior to neurosurgical intervention, particularly in brain tumor patients and patients with other conditions such as epilepsy.

Peer review

The manuscript is well written and authors are reporting a valuable research. Methods and material section is well described and results are defined well.

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Events Calendar 2011

January 13-14, 2011
3rd Breast-Gynecology International Cancer Conference BGICC, Cairo, Egypt

January 15-16, 2011
Melanoma 2011: 21st Annual Cutaneous Malignancy Update, San Diego, CA, United States

January 15, 2011
Current Trends in Breast Cancer: Updates From the 2010 San Antonio Breast Cancer Symposium, Dallas, TX, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA, United States

January 21-23, 2011
8th Meeting of the EAU Section of Oncological Urology, London, England, United Kingdom

January 27-28, 2011
2nd National Conference: Recent Advances in Renal and Bladder Cancer, London, United Kingdom

January 27-28, 2011
8th Annual Cancer Drugs Research & Development, San Diego, CA, United States

February 10-12, 2011
17th Annual NOCR Meeting, Las Vegas, NV, United States

February 19-22, 2011
Scripps Cancer Center's 31st Annual Conference: Clinical Hematology and Oncology, San Diego, CA, United States

February 24-26, 2011
European Multidisciplinary Conference in Thoracic Oncology (Lung 2011-EMCTO), Lugano, Switzerland

February 25-27, 2011
7th European Congress on Hematologic Malignancies: From Clinical Science to Clinical Practice, Budapest, Hungary

March 02-05, 2011
64th Society of Surgical Oncology Annual Cancer Symposium 2011, San Antonio, TX, United States

March 04-06, 2011
8th Annual Oncology Nursing Advanced Practice: Innovation through Practice, San Diego, CA, United States

March 07-09, 2011
9th International Symposium on Targeted Anticancer Therapies, Paris, France

March 09-13, 2011
16th National Comprehensive Cancer Network Annual Conference (NCCN 2011), Hollywood, FL, United States

March 11-12, 2011
12th European Congress: Perspectives in Lung Cancer, Torino, Italy

March 14-18, 2011
Oncology Imaging Update in Costa Rica, Guanacaste, Costa Rica

March 17-19, 2011
International Cancer Prevention Update Symposium, New York, United States

March 18-22, 2011
Vienna, Austria 26th Annual EAU Congress

April 02-06, 2011
AACR 102nd Annual Meeting, Orlando, FL, United States

April 08-10, 2011
Asian Oncology Summit 2011, Hong Kong, China

April 20-23, 2011
9th International Gastric Cancer Congress, Seoul, South Korea

April 29-30, 2011
Cancer Survivorship Conference, Minneapolis, MN, United States

May 23-24, 2011
4th International Conference on Ovarian Cancer Screening, London, United Kingdom

June 03-07, 2011
47th American Society of Clinical Oncology Annual Meeting, Chicago, IL, United States

June 20-23, 2011
7th EADO Congress European Association of Dermato-Oncology, Nantes, France

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

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"MASCC/ISOO 2011 International Symposium, Athens, Greece

July 03-07, 2011
14th World Conference on Lung Cancer, Amsterdam, Netherlands

July 14-17, 2011
3rd World Congress of the International Academy of Oral Oncology 2011, Singapore, Singapore

August 15-17, 2011
International Conference and Exhibition on Cancer Science & Therapy, Las Vegas, Nevada, United States

September 1-3, 2011
Tri-Society Head and Neck Oncology, Singapore, Singapore

September 7-10, 2011
Hallmarks and Horizons of Cancer, Lausanne, Switzerland

September 23-27, 2011
Joint 16th ECCO and 36th ESMO Multidisciplinary Cancer Congress, Stockholm, Sweden

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November 30-December 03, 2011
AORTIC 2011-Entering the 21st Century for Cancer Control in Africa, Cairo, Egypt

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INSTRUCTIONS TO AUTHORS

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Acknowledgments

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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Patent (list all authors)

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Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Italics
Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
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