Role of cyclooxygenase-2 in cervical cancer

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Abstract

Concurrent radiation and chemotherapy is a standard treatment of patients with cervical cancer in FIGO stages IB2-IVA. Chemotherapy given in the same time as irradiation improved survival significant, but not involves all patients. Cancer recurrence is the most common treatment failure in patients with advanced tumor. Further improvement in the treatment of advanced cases is essential. The increasing knowledge about new biologic markers and better ability to predict risk of cancer recurrence is very important for construction of more effective treatment strategies. Currently, there is considerable interest of role of cyclo-oxgenase-2 in cervical neoplasia. A lot of cancers in different localizations appear to overexpress the cyclooxygenase-2, which may have an important role in carcinogenesis. Ongoing clinical trials, clinical studies have shown the positive therapeutic effect of COX-2 inhibitors and may allow to increase understanding of this novel targeted approach for cervical cancer control.

Key words: cervical cancer, radiochemotherapy, cyclooxygenase-2.

Introduction

Irradiation associated with chemotherapy (radiochemotherapy) is a standard treatment of loco-regional advanced cervix cancer. Despite overall and disease free survival improvement (on 12 and 18% respectively), cancer recurrence has been observed in a significant percentage of patients. Further improvement in the treatment of advanced cervix cancer is desperately needed. FIGO stage, lymph nodes status, tumor size, hemoglobin level are well recognized prognostic factors for radio-chemotherapy results, but in some patients with the radioresistance tumor, aggressive clinical course treatment results were not always correlated with the mentioned factors. Recognition of biological tumor markers allowing for better treatment failure risk prediction is very important in developing of new therapeutic methods and better patients selection for different methods of treatment.

For fifteen years the role of cyclooxygenase-2 (COX-2) in carcinogenesis and tumor progression has been a subject of a lot of research. Cyclooxygenase enzyme exist in two main isoenzyme forms (Figure 1). COX-1 is expressed in most of tissues and catalyzes the synthesis of prostaglandins from arachidonic acid, which are required for normal, physiologic functions e.g. gastrointestinal cytoprotection and platelet activity, it is also expressed in endothelial cells, renal microvasculature [1, 2]. COX-2 is not detectable in most normal tissues, and basal conditions. It is induced by cytokines (inflammatory response), growth factors, tumor promoters and then COX-2 is overexpressed in many cell types like

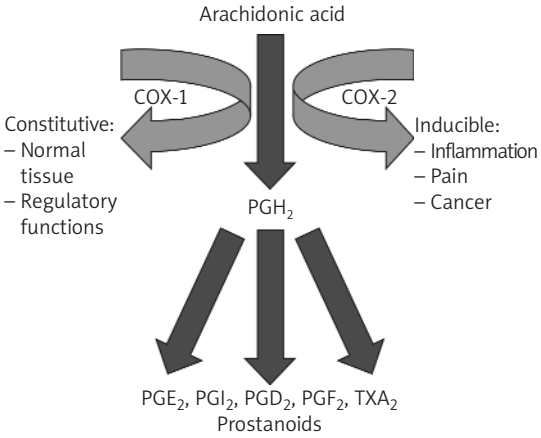

Figure 1. Synthesis of prostaglandins and tromboxans from arachidonic acid

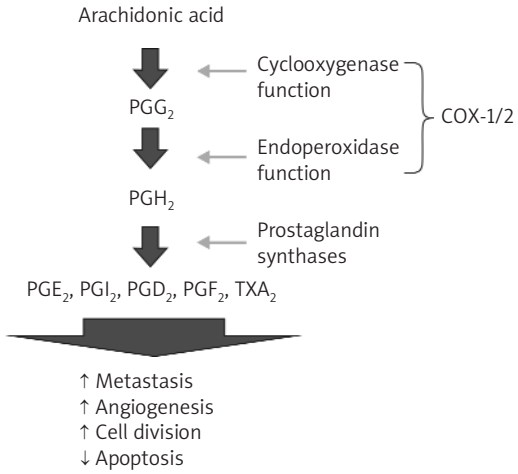

Figure 2. The role of cyclooxygenase-2 (COX-2) in cancer development

macrophages, epithelial, endothelial cells, fibroblast and thus contributes to increased prostaglandins synthesis in inflamed and neoplastic tissues [2, 3]. COX-2 overexpression was observed in early carcinogenesis stages in colon cancer and carcinogenesis suppression was observed in mice disable of COX-2 gen. COX-2 overexpression has been noticed in different types of cancer including pancreatic, lung, breast, colorectal, esophageal, gastric, bladder, ovary, endometrial and cervix cancer [4-6].

Experimental evidence of a role of COX-2 in carcinogenesis

COX-2 contribution in carcinogenesis may go through a different mechanisms, including procarcinogens activation like benzopyren found in tobacco and grilled foods [7], increase of cancer cell invasiveness, inhibition of apoptosis, immunosupression and stimulation of angiogenesis (Figure 2). Inhibition of apoptosis has been observed in rat intestinal cells: overexpression of COX-2 lead to increase the anti apoptotic protein Bcl-2 level and to suppression of apoptosis and allow to survival of cancer cell [8]. The invasive potential of human colon cancer cells may increased when COX-2 is overexpressed: it is associated with activation of metalloproteinase-2, which promote invasion. COX-2 induce prostaglandin E_2, which suppresses humoral and cellular immune response and stimulates immunosuppressive cytokines. This effect can be reversed by COX-2 inhibition, as it was observed in a murine Lewis lung carcinoma model [8-10].

In experimental conditions on colon carcinoma cell line angiogenesis inhibition by the selective COX-2 inhibitor NS 398 has been proven [11].

COX-2 and irradiation in cell cultures

The mechanism of COX-2 inhibited radiosensitivity is not completely understood. Steinauer *et al*. demonstrated that COX-2 can be blocked by the use of a specific inhibitor before radiotherapy [12]. In experimental cell lines an enhancement of tumor cell radiosensitivity by selective inhibitor of COX-2 has been shown in murine sarcoma cell culture, human cancer cell lines, and in rat intestinal epithelial cells COX-2 overexpressing [13, 14]. This increasing radioresponse may be attributable to enhancement of radiation-induced apoptosis, accumulation of cells in the radiosensitive G2-M phase of the cell cycle and inhibition of sublethal radiation damage repair. The radiation-induced G2-M arrest by using selective COX-2 inhibitor was observed also in the COX-2 low expressing cells, caused by another not very understanding mechanism. Selective COX-2 inhibitor showed synergistic with irradiation antitumor activity without increasing radiation damage to normal tissue in an sarcoma cell line model.

COX-2 and cytostatics in cell cultures

Interaction between COX-2 inhibitors and cisplatin and paclitaxel has been investigated in non-small cell lung cancer and small cell lung cancer *in vitro*: sulindac, the non specific COX-2 inhibitor enhanced growth inhibition of cytostatics [15]. In the same cell lines induction of apoptosis has been observed when a selective COX-2 inhibitor was given with cisplatin, etoposide, irinotecan, docetaxel. The synergistic effects between chemotherapy and COX-2 inhibitors may be considered in the treatment [16].

OX-2 and HPV

The role of human papilloma virus oncoproteins E6 and E7 in cervix cancer genesis have been well known. The effects of virus proteins E6 and E7 on *COX-2* expression are unknown. In experimental condition increased levels of COX-2 mRNA, protein,

and prostaglandin E_2 were detected in HPV16 E6- and E7-expressing cervical cancer cells culture compared with HPV-negative cervical cancer cell line. HPV16 oncoproteins stimulated EGFR, induced also some coactivators/corepressor and in this way induced COX-2 transcription. Munoz *et al*. [17] and zur Hausen *et al*. [18] have shown that HPV may play role in induction of COX-2. Kim *et al*. [19] suggested that COX-2 overexpression was not correlated with HPV positivity. Molecular changes caused by HPV may not affect the synthesis of COX-2 in cervical cancer cells. Similar conclusion have been made by Song *et al*. [20].

COX-2, cervical cancer and clinical studies

The increasing knowledge of proteins, which physiologic levels use to be disturb in time of carcinogenesis and cancer progression and development of new immunohistochemical techniques have lead to use biomarkers as a prognostic and/or predictive factors. The expression of COX-2 has been detected in cervical intraepithelial neoplasia (CIN) and in cervical cancer tissue. Most authors suggested, that COX-2 is undetectable in normal cervix tissue. A very sparse suggested, that normal cervical tissue expressed COX-2 more frequently than cervical cancer, but their clinical material concerned a very small groups of patients [21].

A few studies tested correlation between COX-2 expression and pathological parameters in cervix cancer. The relationship between tumor grade and COX-2 expression is not very clear. Both low grade of histological differentiation and high COX-2 expression are accepted as a poor prognostic factors, but Chen *et al*. [22] observed inverse relationship: the expression of COX-2 in grade I tumor was significantly higher compared to grade II and III. Chen put hypothesis, that COX-2 expression is important first of all during carcinogenesis and later its role might be not very significant. Ferrandina *et al*. [23], Lee *et al*. [24], Dai *et al*. [25] did not observe differences with respect to tumor grade and COX-2 expression. These results suggest, that COX-2 is not involved in tumor grade determination or, that COX-2 expression might be reduced in the presence of aggressive cellular differentiation.

Kim *et al*. [26], Ryu *et al*. [27] have shown, that COX-2 overexpression correlated with stage, risk of lymph nodes metastasis and parametrial involvement. Similar results have been shown by Pyo *et al*. [28] and Ferrandina *et al*. [23]: they attributed this correlation to a direct association high COX-2 level and advanced tumor stage and tumor size. Some of them suggest more important role of COX-2 expression in local tumor spread (also on context of risk of lororegional recurrence) than in nodal metastases. Others, could not demonstrate any correlation between COX-2 expression and parametrial invasion and/or lymph node metastases [22, 29]. Dursum *et al*. [30] observed significantly higher expression of COX-2 in patient with cervix tumor size > 4 cm and with lymphovascular space invasion (LVSI). The same relationship between COX-2 level and LVSI was observed by Chen *et al*. [22] and Lee *et al*. [24]. Some researches could not find any dependence between COX-2 expression and cervix cancer tumor size [22, 31].

Relationship between HPV status and COX-2 expression is analyzed in a few clinical reports. HPV infection may play an important role in stimulation of COX-2 as it was shown by Munoz *et al*. [32] and zur Hausen *et al*. [33]. On the other hand Kim *et al*. [19] and Kulkarni *et al*. [6] did not find correlation between COX-2 expression and HPV positivity. This lack of relationship may be caused by different pathways in cervical carcinogenesis, or molecular changes caused by HPV may not have influence on COX-2 overexpression.

The analysis of radiotherapy and radiochemotherapy results in patients with cervical cancer indicate that high COX-2 expression may be correlated with lower survival. Kim *et al*. [31] noticed significantly higher incidence of local failure for patients with high COX-2 expression then for patients COX-2 negative. Pyo *et al*. [28], Kang *et al*. [34], Ferrandina *et al*. [35, 36], noticed decreased survival in patients with elevated COX-2 expression. Ferrandina *et al*. [37] in the group of 175 patients with different stage of cervix cancer confirmed that COX-2 status in both tumor and stroma compartment (ratio) can help in identification of cervix cancer patients with low probability of response to neoadjuvant chemotherapy and preoperative chemoradiotherapy.

The mechanism by which COX-2 is up-regulated in cervix cancer is not very clear. Kulkarni *et al*. [6] suggest first deregulation of EGFR signaling pathway and then COX-2 increased expression. Most of authors concentrated on aftermath of COX-2 expression. In Stolina *et al*. [10] study COX-2 expression is correlated with antagonize host immunogenity against cancer cells, similarly Chen *et al*. [38] suggested that high COX-2 level might be important in inhibiting host immune system (indicated by lower tumor intraepithelial CD8+ lymphocyte count) which is poor prognostic factor for patients. Ryu *et al*. [39] have shown in group of cervix cancer patients treated by radical surgery that expression of COX-2 may downregulate apoptosis and in this way enhance invasion and metastases. Nagai *et al*. [40] find interesting correlation between lack of COX-2 expression and significant induction of apoptosis during neoadjuvant chemotherapy. In patients with COX-2 overexpression the difference in apoptotic index before and after chemotherapy is not considerable.

Initially COX-2 level may be a predictor of response of chemotherapy. It has been documented by better pathological response to chemotherapy in patients with COX-2 protein negative. The same reported Ferrandina *et al*. [35]. Ishikawa *et al*. [41] prospectively assessed apoptotic index in specimens taken before and during radiotherapy (after the dose of 9 Gy) and they find significant negative correlation between initial COX-2 expression and apoptotic index during treatment. Complete response rate was 80% for COX-2 negative patients and 59% for patients with COX-2 overexpression. The 2-year local control was statistically better for COX-2 negative patients. Level of COX-2 expression before radiotherapy may help to predict response for treatment. Concurrent chemotherapy had no impact on apoptotic index measured during radiotherapy probability because of a limited effect of low dose of cisplatin.

COX-2 inhibitors, cervical cancer and clinical studies

A lot of epidemiologic, experimental and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) have anticancer activity. This group of drugs have anti-inflammatory effects due to inhibition the synthesis of prostaglandins, but prostaglandins mainly E_2 have also the important role during carcinogenesis, and the inhibition of prostaglandins formation by blocking COX-2 may protect against many types of cancers like breast, colon, head and neck, skin, cervix and ovary cancer.

Rao *et al*. [42] showed that indomethacin, non specific COX inhibitor protects against chemically induced cervical cancer in mouse. Recently Ferrandina *et al*. [43] in pilot study have shown that treatment with celecoxib, a selective COX-2 inhibitor patients with cervical cancer could affect important aspects of tumor biology: prostaglandins E_2 level, microvessel density, apoptosis level.

Gaffney *et al*. [44] in phase II study of acute toxicity for Celebrex and chemoradiation in patients with locally advanced cervix cancer found a high incidence of acute reactions. Most frequent toxicities were hematologic, gastrointestinal, skin.

Herrera *et al*. [45] in a prospective phase I-II trial of COX-2 inhibitor Celecoxib in patients with cervix cancer observed higher than expected late complications mainly rectovaginal fistula. There are some limitations in these two studies with regard to the effectiveness of celecoxib in cervical cancer treatment: pretreatment COX-2 expression was not examined. The most effectiveness of COX-2 inhibitors would be expected in COX-2 overexpressing tumors. The toxicities of NSAIDs include gastrointestinal bleeding, renal toxicity, inhibition of platelet function. COX-2 selective inhibitors newer generation (valdecoxib, parecoxib) are more safety particularly in gastrointestinal tract. In cancer treatment it is very important to consider agents that are effective and have minimal toxicity. Some reports suggest that COX-2 inhibitors increase risk for stroke, myocardial infarction up to 3.7 fold compare with placebo, some authors conclude that risk is dose-related. On the other hand, on most *in vitro* studies the COX-2 inhibitors concentration in cancer tissues that was correlated with optimal results was higher than 35 µmol/l [46, 47]. This suggest higher doses may be needed for significant antitumor effect. According to these results, if proper selection of COX-2 inhibitor, the smallest effective dose giving in as short as possibly time during radiotherapy could be recognized, more effective treatment for advanced cervix cancer may be defined.

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