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TPL-2/COT AND COX-2 IN BREAST CANCER

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Background: Breast cancer is the most common cancer in women worldwide and although mortality (129 000/year) stagnates, incidence (370 000/year) is increasing. In addition to histological type, grade, stage, hormonal and c-erbB2 status there is therefore a strong need for new and reliable prognostic and predictive factors.

Methods and results: This minireview focuses on two potential prognostic and predictive candidates Tpl2/Cot and COX-2 and summarise information about them.

Conclusion: Tumor progression locus 2 (Tpl2/Cot) is a serine/threonine protein kinase belonging to the family of MAP3 kinases. Activated Tpl2/Cot leads to induction of ERK1/2, JNK, NF-κB and p38MAPK pathways. The first study on Tpl2/Cot mRNA in breast cancer showed its increase in 40 % of cases of breast cancer but no available data exist on protein expression. Cyclo-oxygenase 2 (COX-2) is inducible by growth and inflammatory factors and contributes to the development of various tumours. Expression of COX-2 in breast cancer varied from 5-100 % in reviewed papers with significantly higher values in poorly differentiated tumours. Tpl2/Cot and COX-2 have their importance in different intracellular pathways and some of these are involved in cancer development. Briefly, the results from recent studies suggest that Tpl2/Cot and COX-2 could be prognostic factors in breast cancer.

INTRODUCTION

Breast cancer is the most common cancer type in women worldwide. The incidence of this disease was approximately 370 000/year in Europe from 2000 to 2005. This includes 27.4 % of all cancer types. With 129 000 deaths/year (17.4 %) it is the most common cause of cancer death in women[1]. The incidence and mortality in Czech Republic is similar to the rest of Europe: breast cancer is the cause of every fourth death and although the incidence is increasing, mortality stagnates and in some countries decrease was registered[2, 3]. According to WHO classification, the largest group of breast carcinoma is ductal carcinoma (80 %), followed by 12 % of lobular carcinoma, 8 % covers rare tumours such as tubular carcinoma, Paget's carcinoma, sarcoma or lymphoma[4]. Recently a new classification has been proposed according to the molecular expressing profile and application of DNA chip arrays. Breast carcinomas are divided into the four subtypes: luminal subtype, normal basal-like subtype, HER2 positive subtype and basal-like breast cancer[5] with varying sensitivity to tamoxifen, etoposide and 5-flourouracil in particular groups. It is clear that assessment of histological type is only one part of the process of establishing a diagnosis, prognosis and prediction of these tumours and as many reviews make clear[5–7], in addition to histological type, grade, stage, hormonal and c-erbB2 status, new specific markers which could aid in the prognosis and prediction of breast cancer are still needed.

Recently, the inflammatory molecules have emerged as important in the development of cancer - inflammatory mediators are in large measure responsible for the events leading to the formation, growth and metastasis of tumours. This has been widely reviewed by Aggarwal et al.[8]. This minireview is focused on two potential candidates, Tpl2/Cot and COX-2 which seems to play a particular role during progression and metastasis of breast cancer[9].

TPL-2

Tumor progression locus 2 or Tpl2/Cot is a serine/threonine protein kinase belonging to the family of mitogen-activated protein 3 kinases. It was first identified in T-cell lymphoma induced by MoMuL (Moloney murine leukaemia) virus and in breast carcinoma induced by MMT (mouse mammary tumour) virus[10]. In non-stimulated cells, Tpl2/Cot is inactive and exists in a complex with p105 regulation subunit also known as NF-κB1 (ref.[10, 11]), which is a negative regulator of Tpl2/Cot activity[12]. Phosphorylation of p105 on its C-terminal region and Tpl2/Cot phosphorylation at Thr290 leads to its release from p105 and subsequently, a further phosphorylation of Tpl2/Cot on Ser62 via IL-1 stimulated protein kinase leads to its full activation[13-16].

Tpl2/Cot can be activated by bacterial lipopolysachide (LPS), an endotoxin derived from the wall of Gram-negative bacteria[17]. For instance, Tpl2/Cot endogenous activity increases 10-fold in macrophages after LPS stimulation[18]. Activation of Tpl2/Cot via LPS is mediated by transmem-

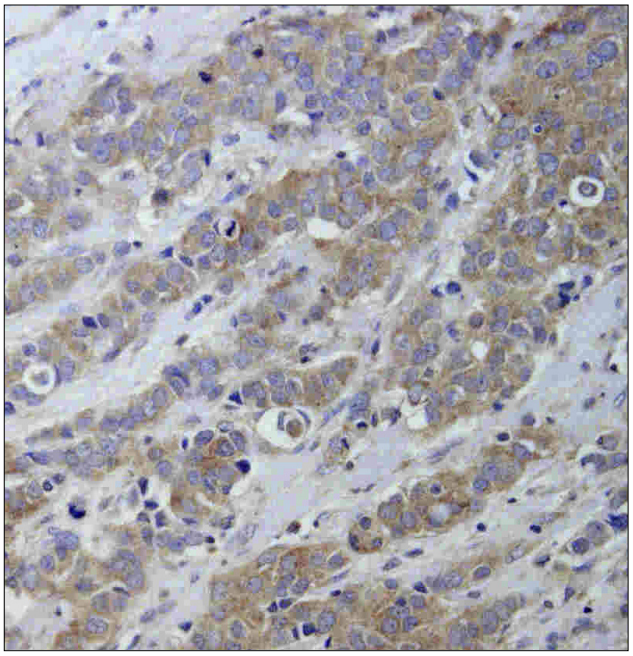


Fig. 1. Moderate to high cytoplasmic expression of Tpl2/Cot in invasive ductal breast carcinoma. Magnification x 200. (Anti –TPL2 antibody; rabbit-polyclonal; dilution 1 : 50; Santa Cruz)

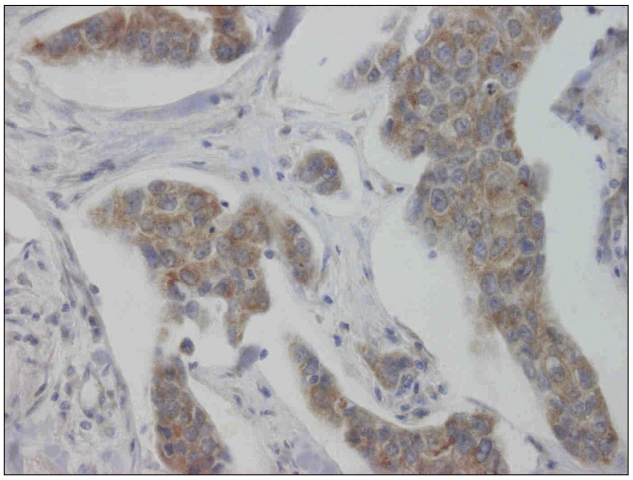


Fig. 3. Moderate to high cytoplasmic expression of COX-2 in invasive ductal breast carcinoma. Magnification x 200. (Anti-COX-2 antibody; rabbit-polyclonal; dilution 1:50; Santa Cruz)

brane receptor TLR4 (Toll-like receptor)[19]. Interestingly, activation of Tpl2/Cot is achieved through similar mechanisms by paclitaxel[18]. Kelly et al. have found a relation between activation of TLR4 signalling pathway and chemoresistance to paclitaxel therapy in ovarian cancer. Hence if this correlation is found in breast cancer, then changes in expression of TLR4 or increased activity of Tpl2/Cot could predict inefficiency of taxane-based treatment[20, 21].

Tpl2/Cot is part of several intracellular signalling pathways and for some of them its role is pivotal. Over-

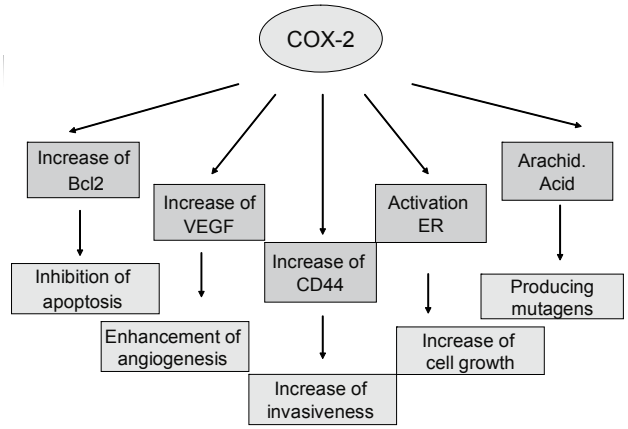


Fig. 2. Contribution of COX-2 to the carcinogenesis.

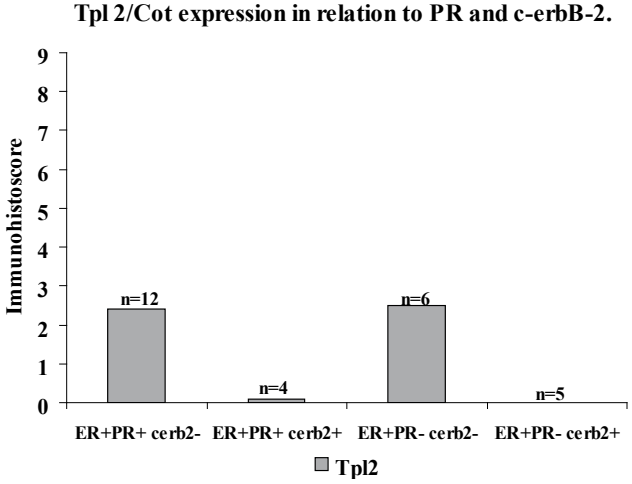
expression of Tpl2/Cot leads to activation of ERK1/2, JNK, NF-κB and p38MAPK pathways[17, 22]. ERK activation via Tpl2/Cot is based on TRAF6 and TRAF2 association with CD40 (member of the TNF superfamily) and subsequent Tpl2/Cot binding with TAK1/TAB complex, which results in induction of NF-κB[23-26]. Another role of Tpl2/Cot is negative regulation of Th1-type adaptive immunity by inhibiting IL-12 production[27] and induction of RANKL in response to synthetic lipid A in osteoblasts[28]. Tpl2/Cot also affects the subcellular distribution of the COX-2 message and promotes stabilization of this protein in cells[29].

Negative regulators of Tpl2/Cot activated ERK1/2 pathway are arrestin-2 and GRK5 (G protein-coupled receptor kinase 5). Arrestin-2 binds directly to the COOH-terminal region of p105 and this blocks phosphorylation and release Tpl2/Cot from p105. GRK5 also directly interacts with p105 (ref.[30]).

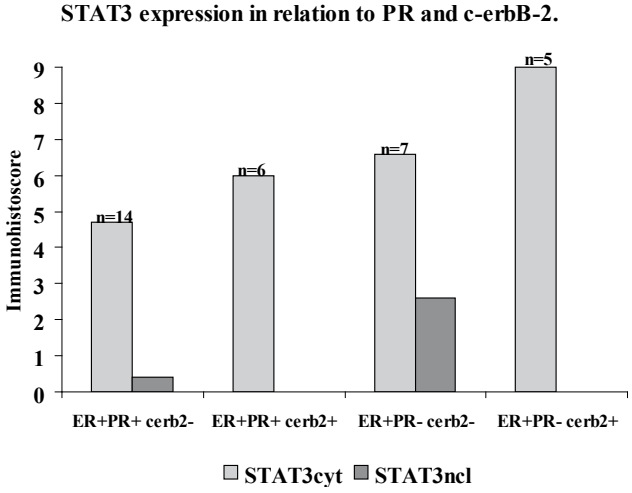
The first studies on experimental inhibition of Tpl2/Cot demonstrated the importance of this molecule. Quinolin-3-carbonitril was identified as a potent and selective Tpl2/Cot inhibitor in rheumatoid arthritis[31]. 15-Deoxy-Δ12, 14-prostaglandin J2, a potent natural ligand of PPAR-γ, also completely suppressed the activation of Tpl2/Cot activated by LPS or Paclitaxel[18].

Activation of Tpl2/Cot has been shown both in various inflammatory processes such as pancreatitis and rheumatoid arthritis and in tumours such as T-cell neoplasias, breast cancer cell lines, etc.[10-36] .

The first study focused on Tpl2/Cot mRNA in breast cancer tissues showed its overexpression in 40 % of cases, and significant correlation to Tpl2/Cot gene amplification[32]. Moreover, positive significant association with TNM stage I was identified in the same study, indicating that this molecular alteration may be an early event in the development of the disease. It was also suggested, that overexpression of the progesterone receptor correlates



Graph 1. Significant increase of Tpl2/Cot expression in c-erbB-2 negative carcinomas ($p \leq 0{,}001$).



Graph 2. Significant increase of STAT3 nuclear expression in c-erbB-2 negative carcinomas ($p \leq 0{,}014$).

with overexpression of Tpl2/Cot[32], but larger studies, to elucidate its relation to oestrogen and progesterone receptors, Her2/neu and histological features do not exist to date. Our preliminary results from immunohistochemical study on tissue microarrays (TMA) of 32 cases of invasive ductal carcinomas showed significant increase in Tpl2/Cot expression in c-erbB-2 negative carcinomas[33] ($p \leq 0{,}001$, Fig. 1, Graph 1). Interestingly, this increase also correlated with enhanced nuclear expression of STAT3, another molecule involved in immunity and inflammation, in the same group of tumours ($p \leq 0{,}014$, Graph 2). The potential predictive value of increased Tpl2/Cot and STAT3 expression is supported by the finding that inhibition of STAT3 increases response to the proapoptotic effects of doxorubicin in breast cancer cells (MDA-MB-231)(ref.[34]). Moreover, another STAT family member, STAT5b, showed a relation to the induction of differentiation in breast cancer cells transfected with progesterone DNA[35]. For this reason, in poor prognosis patients without expression of progesterone receptor, STAT5b may also play prognostic role – its absence may contribute to poor prognosis. Taken together, despite the small size of this studied series, these results indicate that increased Tpl2/Cot and STAT3 expression in c-erB-2 negative breast cancer tumours could by another important prognostic and predictive factor.

COX-2

Cyclo-oxygenase 2 (also prostaglandin endoperoxide synthase 2), is the most extensively studied proteins in human cancer: COX-2 is inducible by growth factors and inflammation stimuli and as has been described in several reviews[37-40] COX-2 contributes to the development of various types of tumour[41-43].

In breast cancer COX-2 play role in these processes[37-40] (Fig. 2):

- inhibition of apoptosis by induction of PGE2, which leads to increased expression of antiapoptotic protein BCL-2 and decreased expression of proapoptotic protein BAX and to weakening of nitric oxide (NO) signals
- enhanced angiogenesis due to increased PGE 2 level, followed by increased VEGF, endothelin-1 and PDGF production
- increased invasiveness via overexpression of CD44
- increased cell growth via oestrogen receptor activation
- producing mutagens by metabolism of arachidonic acid

Howe and Dannenberg found that elevated levels of COX-2 protein correlate with tumour size, high proliferation rate, axillary node metastases, histology, human epidermal growth factor receptor 2 (HER-2) gene amplification and decreased disease-free survival in breast cancer[40]. Association of high COX-2 expression with reduced disease-free survival and also with disease-related survival was also found in oestrogen receptor (ER) negative breast cancers[44]. Expression of COX-2 in cancer cells varied from 5 % to 100 % in reviewed papers with an average score of 40 %[38, 40, 44]. COX-2 expression was found both in invasive and in *in situ* breast cancer[43] and, in poorly differentiated carcinomas, the intensity of expression was significantly higher[45]. Adjacent, non-neoplasmic tissues were negative for COX-2 staining[45]. As in these studies, our results on tissue microarrays (TMA) of 32 cases of invasive ductal carcinomas show diffuse strong cytoplasmatic, granular expression of COX-2 in all studied tumors (Fig. 3)(ref.[33]).

The role of COX-2 gene polymorphism in breast cancer development is also a matter of current discussion. Women homozygous for *T allele* at 5275 had a 20 % lower risk of breast cancer than those with homozygous for *C allele* [46].

COX-2 is also a target for therapy by selective (celecoxib) or non-selective non-steroid anti-inflammatory drugs (aspirin, indomethacin) in several diseases and their protective contribution against the development of

various tumour types has been shown in animal mouse and rodent models[38]. Studies on rodent breast cancer have shown a significant decrease in the incidence, multiplicity and volume of tumour after selective NSAIDs treatment[38, 40].

The correlation between Her2/neu and COX-2 is also interesting; in celecoxib-treated mice with positive Her2/neu the progression of breast tumours was significantly lower. The blood levels of celecoxib in mice were within the range reported to inhibit inflammation[47]. When COX-2 knockout mice with the same type of breast tumours were investigated, similar results such as decrease in PGE2 production, decreased multiplicity of tumours, decreased angiogenesis and smaller size of lesions, was observed[48]. Howe and Dannenberg[38-40] summarized a large number of studies and suggested that COX-2 inhibitors could be a good target not only for treatment but also for the prevention of breast cancer.

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