Clinicopathologic Significance of Cyclooxygenase –2 mRNA Expression in Human Breast Carcinoma

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Abstract **Objective** To study the expression of cyclooxygenase-2 (COX-2) gene in breast cancers and its relationship to clinicopathologic characteristics. **Methods** With β-actin gene as reference, the COX-2 mRNA was monitored in 30 cases of breast cancer tissues and adjacent normal breast tissues by reverse transcription-polymerase chain reaction(RT-PCR). **Results** The COX-2 mRNA expression was significantly upregulated in most human breast cancers with range of 0.05-0.91(median 0.56), which was rare in normal breast tissues with range of 0-0.09(median 0). The difference of expression of COX-2 mRNA between cancer and nomal breast tissue was significant(rank sum test, $P<0.05$). COX-2 overexpression in breast cancer was related to lymph node metastasis($P<0.05$), but not to age, tumor size, pathologic grading or pathologic type($P>0.05$). **Conclusion** The level of COX-2 mRNA expression is obviously higher in the breast cancer tissue than in normal breast tissue. COX-2 overexpression may play a crucial role in the carcinogenesis, development of cancer and lymph node metastasis in breast cancer patients.

Key Words cyclooxygenase; gene expression; mRNA; breast neoplasms/pathology; RT-PCR

Cyclooxygenase(COX), is a rate-limiting enzyme in the biosynthesis of prostaglandins(PGs) and related eicosanoids. Two isoforms of COX have been identified and cloned in eukaryotic cell. COX-1 is constitutively expressed in most cell types and is thought to be involved in the maintenance of physiological functions. In contrast, COX-2 is inducible by proinflammatory cytokines, tumor promoters, mitogens, oncogenes, and growth factors in various cell types such as monocytes, fibroblasts, smooth muscle cells, and endothelial cells. Dysregulation expression of COX-2 gene is correlated with the pathogenesis of inflammatory diseases, developmental events and tumorigenesis. In recent studies, COX-2 protein and gene overexpression have been detected in colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, pulmonary carcinoma, esophageal carcinoma, hepatic carcinoma, prostate carcinoma. But rare report concerns COX-2 mRNA expression in the breast cancer tissues. Now we will report our study as follows.

MATERIALS AND METHODS

Patient Samples

All of 30 primary breast cancer patients are female, and mean age is 55. The samples were collected from July, 2001 to January, 2002 and contained breast cancer tissues and normal breast tissues which are more than 7 cm far away from the cancer tissues. The samples were rapidly frozen in the liquid nitrogen, then conserved at -80℃.

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RNA isolation and RT–PCR

Total RNA from each sample was isolated using TRIzol Solution (Gibco Inc., USA). RNA concentration and purity were determined by absorbance at 260 and 280nm. Four micrograms of total RNA were electrophoresed through 2% agarose gel and intergrity of the RNA was monitored by ethidium bromide staining.

According to the manufacture's instructions, the reverse transcription and subsequent amplification of the cDNA sequence were performed by using the TITANIUMTM one-step RT-PCR kit (Clontech Inc. USA). Five micrograms of total RNA was used for RT-PCR and β−actin was used as quantitative control. Primer were designed as follows: COX-2: 5'-AAGCCTTCTCTAACCTCTCC-3', 5'-TAAGCACATCGCATACTCTG-3', the length of amplification product is 531bp; β−actin: 5'-GTTTGAGACCTTCAACACCCC-3', 5'-GTGGCCA-TCTCTCTTGCTCGAAGTC-3', the length of amplification product is 320bp. The RT-PCR was performed in a thermal cycler (Eppendorf Co., Germany) under the following conditions : (1) reverse transcription reaction at 50℃ for one hour (2) inactivate MMLV reverse transcriptase at 94℃ for 5 min (3) amplification of the cDNA by 30 cycles of 30s at 94℃, 30s at 55℃ and 1 min at 68℃ (4) a final extension step of 2 min at 68℃.

Amplification mixture was subjected to electrophoresis on 2% agarose gel, and DNA was visualized

by ethidium bromide staining. The signal intensity was quantified by a computerized image-processing system (Bandscan). The ratio of COX-2 to β-actin was used to express relative mRNA levels.

Statistics

These data take on the skew distribution, and rank sum test is used to analyse the data of optical density of carcinoma tissues and normal tissues (Wilcoxon pairing rank sum test) and the relation between COX-2 gene expression and age, tumor size, lymph node metastasis, pathologic grading as well as pathologic type(Wilcoxon grouping rank sum test).

RESULTS

The expression of COS-2 mRNA was significantly upregulated in ninety percent (28/30) of breast cancers with range of 0.05-0.91(median 0.53). However, no expression or slight expression was observed in 30 normal breast tissues, with range of 0-0.09 (median 0). Though rank sum test, there was significant difference between breast cancer tissues and normal breast tissues (Fig. 1) ($P<0.05$).

The COX-2 high expression is relevant to the breast cancer lymph nodes metastasis ($P<0.05$), but it did not correlate with age, tumor size, pathologic grading and pathologic type($P>0.05$)(Table 1).

DISCUSSION

Regarding the expression of COX-2 in breast cancers, to date, several investigations mainly focus on the expression level of COX-2 protein or using breast cancer cell lines to analyse[1]. Few study concerns the expression of COX-2 mRNA in breast cancer tissues. Our study showed that the expression COX-2 mRNA was significantly upregulated in most human breast cancers, while there was no or very weak expression in the normal breast tissues. As COX-2 specific inhibitor can restrain the tumor cell growth and the function of preventing the colorectal cancer showed in the epidemiological studies, its action on the tumor occurrence and development had been paid close attention recently. But its concrete mechanisms during the course are unknown now. Study indicated that the mechanisms with which COX-2 and its production PGs act on the tumor might be: (I) To induce cells to proliferate, restrain cells from apoptosis and activate the oncogene to express[2]. The

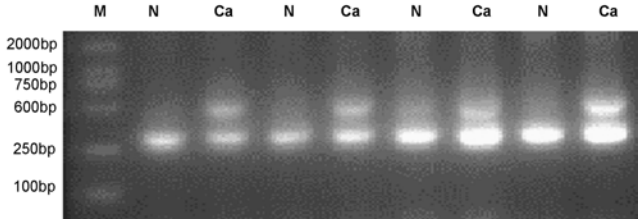

Fig.1 M: Marker Ca: Carcinoma N: Normal tissue
RT-PCR production in sepharose under ultraviolet ray

Table 1 Relationship between clinicopathologic characteristics and COX-2 mRNA expression

clinicopathologic characteristics	n	range	*T* value	P
Age(years)				
<55	14	0.05-0.91	194	>0.05
≥ 55	16	0.07-0.88		
Tumor size(cm)				
<2	12	0.05-0.74	160	>0.05
≥ 2	18	0.09-0.91		
lymph node metastasis				
negative	16	0.05-0.74	172	<0.05
positive	14	0.26-0.91		
TNM grading				
Ⅰ、Ⅱ	19	0.05-0.91	191	>0.05
Ⅲ、Ⅳ	11	0.12-0.83		
pathologic type				
simplex carcinoma	13	0.05-0.88	H=3.74*	>0.05
invasive ductal carcinoma	8	0.09-0.91		
scirrhous carcinoma	5	0.39-0.85		
medullary carcinoma	3	0.07-0.57		
mucoid adenocarcinoma	1	0.26		

Multi-sample rank sum test(Kruskal-Wallis method) is applied to analyse Relationship between clinicopathologic characteristics and COX-2 mRNA expression, whereas medullary carcinoma and mucoid adenocarcinoma are not involved in because of their small amounts

difference between the subcell location of COX-1 and COX-2 leads to the difference of their function. COX-1 was located in the endoplasmic reticulum, while COX-2 in the endoplasmic reticulum and nuclear membrane, so the production of COX-2 catalysis, PGs, can enter endonuclear region and act on the subordinate signal molecular, regulating the cell proliferation and differentiation. PGE2 can activate the II region of aromatase promoter, whose production, estrogens, can promote the mitosis of the breast cancer cells and can restrain the apoptosis of them; (II) PGE2 can stimulate the produce of vascular endothelial growth factor (VEGF), consequently promote angiogenesis[3]; (III) To impair immune surveillance[4]. PGE2 can suppress the activation of NK cells, LAK cells and CTL cells, and the produce of IL-2. These will lead to the decline of the immune surveillance function and the decline of the cell killing ability; (IV) To activate the precarcinogen[5]. The COX-2 protein has the function of peroxidase and can promote the activation of precarcinogen.

It was found that the oncogene ras can up-regulate COX-2 promoter activity, and increase the expression of COX-2 protein and the level of PGE2[6]. Moreover the ras gene mutation is the primary event in the genesis of colorectal cancer. So these show that the COX-2 expression has started in the early stage of adenoma formation, increasing the risk of the tumor generation. Our study indicated that in 30 cancer tissues, the expression level of COX-2 mRNA had no relation with the tumor size and pathologic grading. These indicate that COX-2 has played a role in the early stage during the breast cancer formation. Oshima et al. reported that the mice get rid of gene can lead to familial adenomatous ployposis (FAP), and Ptgs2(mice COX-2 gene) mutation can significantly reduce the number and size of the mice intestinal adenoma. And meanwhile the appliance of the COX-2 special inhibitor can also reduce the mice tumor load [7]. These proved that the COX-2 expression may be induced by some other initiation factors, such as the activation and the mutation of certain genes. The high expression of COX-2 is not enough to induce the formation of the tumor, but can promote the development of the tumor.

There are always high COX-2 expression in the breast cancer with lymph node metastasis and compared with those no lymph nodes metastasis, the difference is significant(P<0.05). So it illustrsted that the COX-2 high expression is relevant to the lymph nodes metastasis in breast cancer. Some studies showed that the COX-2 overexpression can improve the function of metallo proteinases and that may promote the lymph node invasion and the metastasis[8]. Moreover, through the paracrine effect, the cancer cells which express COX-2 can induce the neighbour cells to express COX-2, and then deteriorate, thus, promoting the tumor diffusion[1]. Liu et al. used two human breast cancer cell lines to examine the COX-2 expression. The results showed that the biologically aggressive, invasive MDA-MB-231 cell line, in contrast to MCF-7 cells, possessed a high constitutive level of COX-2[9]. A study showed that the COX-2 overexpression is correlated with the recurrence and metastasis of the tumor[10]. Its value as the tumor marker to evaluate the prognosis is worthy of the further research.

Since some studies showed there are difference between COX-2 mRNA and its protein expression level, it seems likely that COX-2 may undergo complex posttranscriptional and posttranslational modification to yield the active enzyme[9]. So the COX-2 protein expression and its relationship with COX-2 gene expression will require futher study.

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