ORIGINAL ARTICLE

Expression of Estrogen Receptor β in Colon Cancer Progression

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Abstract: Colon cancer is the most frequent neoplasia of the intestine. This pathology is the third highest cause of death from cancer with 430,000 deaths globally per year. Estrogen has also been implicated in the development and progression of colon cancer. Also sex-specific differences have been suggested to be involved in the process. Previous studies have shown the estrogen β receptor to be the dominant receptor type in normal colonic tissue and its down-regulation along with the progression of colorectal cancer. The presence of estrogen receptors and products of estrogen-related genes in the colon suggests that estrogens have direct effects on the colonic tissue. However, the specific effect of estrogens on a normal colon and the role in the colon carcinogenesis are far from clear. The aim of this study is to analyze by real-time polymerase chain reaction, the relative quantitative expression of the estrogen receptors β, β1, β2, and β5 in colon adenocarcinomas and to compare this expression with the respective in normal tissues. Moreover, we evaluate a possible correlation between estrogen's receptor expressions and disease stages. Normal tissues show estrogen receptor β expression greater than pathologic tissues and the estrogen receptor β result as most expressed in the lower disease stages.

Key Words: estrogen receptor, colon cancer, real-time PCR, expression

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Colon cancer (CC) is the third leading cause of cancer deaths with an estimated 430,000 deaths per year world-wide.[1] The prognosis is dependent on the stage of the disease at the time of diagnosis, and the mean 5-year survival rate is relatively poor in most cases.[2] Evidence for a dual role of estrogens in gastrointestinal physiology is accumulating with the high incidence of CC. Age and sex differences in the incidence of gastrointestinal tumors suggest the involvement of sex steroids. The age adjusted incidence rate is higher in men than in women[1] and the protective effect of female hormones is evident in a number of studies. In addition to their effects on sexual development and reproductive functions, the cardiovascular system, the central nervous systems, and bone, both exogenous and endogenous estrogens exert significant effects on gastrointestinal physiology.[3] Estrogens have also been implicated in the development and progression of CC[3] and sex-specific differences have been suggested to be involved in the process.[4] The lifetime risk seems to be significantly lower and the survival rates better[5] in females than in males. Epidemiologic studies have demonstrated that colorectal cancer incidence and mortality rates are lower in women than men.[3] Many studies indicate that estrogen replacement therapy (ERT) exerts a protective role against CC in postmenopausal women.[4] The steroid hormone 17β-estradiol (E2) is a critical regulator of growth, differentiation, and function in a wide range of target tissues. The main biologic functions of E2 are mediated through 2 distinct intracellular receptors, ERα (estrogen receptor α) and ERβ (estrogen receptor β).[5] The ERs mainly act as ligand-activated transcription factors and they modulate gene expression by interactions with promoter response elements or other transcription factors.[6] In addition to this classic ligand-dependent pathway, ER function may also be modulated by extracellular signals in the absence of E2 or a ligand-independent manner.[7,8]

ERs are members of the evolutionary conserved nuclear receptor superfamily of ligand-inducible transcription factors. ERα and ERβ exhibit a modular structure consisting of 6 well-defined functional domains (A–F).[9] Unliganded ERs reside in multiprotein complexes located in the nucleus. Estrogen binding to the "ligand binding domain" induces a conformational change that facilitates receptor homodimerization (ERα/ERα or ERβ/ERβ) or heterodimerization (ERα/ERβ) and high-

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affinity binding to specific DNA recognition sequences [estrogen response elements (EREs)] in the regulatory regions of estrogen target genes. In this "classic" mode of ER action, ERα and ERβ homodimers promote ERE-regulated transcription in response to 17β-estradiol, with ERβ being approximately 30% as efficient as ERα in most cell systems. Estrogens and their cognate receptors

also regulate target genes via a "nonclassic" mode of action. These effects are mediated through promoter elements that bind heterologous transcription factors, including activating protein-1 (AP-1)–binding sites, cyclic AMP response elements (CREs), antioxidant elements and SP-1–binding sites.

Interestingly, ERα and ERβ can exert opposite actions at AP-1 sites in the presence of different ligands. 17β-estradiol increases ERα/AP-1–mediated transcription, but represses ERβ/AP-1 effects, whereas antiestrogens like tamoxifen enhance AP-1–induced transcription through both ERs.[10,11] It is evident that in both the "classic" and "nonclassic" mode of ER action, ERβ, in the presence of estrogen, modulates the proliferating effects of ERα by suppressing transcriptional activation. Thus, ERβ may protect the cell from uncontrolled

All samples were microscopically evaluated and classified as colon adenocarcinoma and normal colonic mucosa.

All tissue pieces were sectioned to ensure that all spatial dimensions were ≤10 mm (10 × 10 × 0.3 mm). All diagnoses were confirmed by examination of 5-μm hematoxylin and eosin-stained sections and all colonic mucosa fragments resulted macroscopically and histologically whole. The fragments were immersed in RNAlater (Qiagen, Milan, Italy) and kept overnight at 4°C and stored at −80°C until analyzed.

RNA Isolation

The tissues (about 5 mg) were defrosted and cut into small pieces. The samples were resuspended in 200 μL