The Synergy of Two Ovarian Hormone-induced Enzymes in Human Mammary Carcinogenesis

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Introduction

The mitogenic activity of estrogens mediated by the estrogen receptor α (ERα) is well established and explains most of the tumor promoter activity of estrogens in breast and endometrium. The basic understanding of the mechanism of trans-activation of ER by agonist and antagonist has led to the first therapies for solid tumors based on the inhibition of estrogen action with antiestrogens and estrogen production with aromatase inhibitors.

By contrast, for progesterone and synthetic progestins, discrepancies between results obtained in cell lines and in patients have slowed down the development of therapies based on the progesterone receptor pathway as a target.

I will review how studies over the last 30 years on the hormone-responsive human breast cancer cell lines have been translated to the clinic with prognostic and predictive markers in response to targeted therapies. A number of studies indicate several candidates among the hormone-responsive genes, some of them closely related to the control of cell cycle. I will concentrate on just two ovarian hormone-induced enzymes, associated with tumor growth and progression, that we have studied extensively, due to their abundance and their specificity of regulation by hormonal steroids. These proteins appear to be less directly and classically related to the initial control of cell cycle than the cyclin-dependant kinases, which are involved in the commitment of cells to enter an active cell cycle, leading to DNA synthesis and mitosis. However, they seem to be required for a tumor to grow and develop as a disease.

I will then summarize our more recent results, obtained in tissues directly collected from patients, concerning the variations in the level of these enzymes and the ovarian hormone receptors in the early steps of breast carcinogenesis.

Estrogens and Cathepsin D

An overview of studies from our laboratory on the hormone-responsive MCF7, ZR75 and T47D human breast cancer cell lines indicates that estrogens, via ERα, trigger a concerted phenotypic program that allows these cells to replicate and divide. Among numerous estrogen-induced proteins, intracellular transcription factors (fos, jun, c myc…) and cyclins (D and E) act as intracrine mitogens to trigger the entry of cells into an active G1 phase of the cycle (Fig. 1). As reported by several authors at this meeting, they directly affect the regulation of the cell cycle. Our laboratory

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In ERα-positive breast cancer cell lines, the levels of different mRNA and proteins have been shown to be stimulated by estrogens and inhibited by synthetic antiestrogens. Estradiol has also been shown to inhibit the synthesis of some proteins (blue sign). Several secreted proteins and peptides (white arrows) are potential paracrine or autocrine mitogens, such as growth factors and some proteases. The 160 kDa protein was not identified. Intra-nuclear proteins (black arrows) are potential intracrine factors. This is the case with cyclin D1, c-myc and c-fos, which are directly involved in the control of cell cycle. (Prog. Rec = progesterone receptor, plasm. activ = plasminogen activator)

has been more interested in estrogen-regulated secreted proteins, different of the classical growth factors, but having also the potential to act as autocrine and paracrine mitogens (Fig. 1). We have focussed on one prevailing, 52-kilodalton (kDa) secreted protein that is specifically induced by estrogens and inhibited by antiestrogens in parallel with the effect of these ER ligands on cell proliferation (Westley and Rochefort 1980; Chalbos et al. 1982; Vignon et al. 1986; Fig. 2). The 52 kDa protein was identified as the precursor of the lysosomal protease, Cathepsin D (cath D), after the quite unexpected discovery that its high level in primary breast cancer tissue extract was associated with an increased risk of clinical metastasis (Spyratos et al. 1989; Rochefort 1996). Cath D was not correlated with ERα in patients since it was also constitutively overexpressed in ER-negative cell lines (Rochefort et al. 1989). Cath D was also induced by growth factors, and its overexpression in human invasive breast cancers was found to be associated in several independent studies with an increased risk of relapse and metastasis (Rochefort et al. 2000; Ferrandina et al. 1997). This enzyme is a good potential therapeutic target because its overexpression stimulates the growth of cell lines and of experimental liver metastasis in vivo when rat embryo fibroblasts are stably transfected with cath D and then injected IV in nude mice (Garcia et al. 1990). Conversely, antisense cath D RNA stably transfected into MDA-MB231 human