《招待講演》

Structure, Function, Regulation and Clinical Significance of the 52 K Pro-Cathepsin D Secreted by Breast Cancer Cells

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Human breast cancers are characterized by their selective responsiveness to estrogen and elevated frequency to metastasize. Micrometastasis can already be present when the primary tumor is removed and it is therefore necessary to discriminate between local breast cancers cured by surgery and generalized breast cancers which would require a general adjuvant therapy aimed at curing the disease. Several prognosis markers are available, the best involves the invasion of axillary lymph nodes. However, about 30% of axillary node negative patients recur, indicating the need for other prognosis markers.

For 10 years, our laboratory has studied the mechanisms by which estrogens stimulate the growth of human metastatic breast cancer cell lines (MCF7, T47D, ZR75-1) in culture. Among the proteins induced by estrogen, we have studied in detail a 52 Kda glycoprotein secreted by MCF7 and ZR75-1 cells whose regulation was associated with cell growth (1). This protein was induced by tamoxifen in antiestrogen resistant cell lines, while being inhibited by tamoxifen in antiestrogen responsive cell lines. The 52 K protein has been purified to homogeneity using monoclonal antibodies and identified as the secreted precursor of a cathepsin D bearing mannose-6-phosphate signals routed to lysosomes *via* mannose-6-phosphate/IGF-II receptors (2,3). The protease is also produced constitutively and in excess by ER-negative cell lines. The corresponding cDNA has been cloned using N-terminal sequencing of the protein and monoclonal antibodies. Its complete sequencing indicates a strong homology with pro-cathepsin D of normal tissues. Using a cDNA probe, the cathepsin D gene and has been localized on chromosome 11 by *in situ* hybridization (4).

In MCF7 cells, cathepsin D is specifically and directly induced by estrogens at the transcriptional level. It is also induced by epidermal growth factor (EGF, IGFI and basic FGF), but this induction depends on *de novo* protein synthesis (5). In hormone dependent and independent breast cancer cells, pro-cathepsin D production and secretion is much higher and its processing is altered, compared to normal mammary epithelial cells, leading to derouting of this protease out of the lysosomes (6). *In vitro*, pro-cathepsin D is an autocrine mitogen (7) on breast cancer cells and can be autoactivated to degrade the extracellular matrix and proteoglycans in some acidic compartment (8). Monoclonal antibodies which recognize the mature form of the protein (34 K chain) allow the assay of total cathepsin D concentration (pro-form 52 K+ mature forms 48 K and 34 K) and the localization of cathepsin D in cells and tissues (9,10). Monoclonal antibodies which recognize only the pro-enzyme 52 K allow the assay and cellular detection of only the pro-cathepsin D (11).

Clinical studies, using both immunohistochemistry of tissue sections or cell aspirates and immunoenzymatic assay of breast cancer cytosol, have shown that total cellular cathepsin D concentration (52 K+48 K+34 K) is related to mammary duct proliferation and to prognosis of breast cancer. Using a two-site solid phase immunometric assay (ELISA or IRMA) cathepsin D concentration can be assayed in cytosol of primary breast cancer, in addition to the steroid receptors (12). Several retrospective clinical studies indicate a significant correlation between high cathepsin D concentrations in the cytosol of primary breast cancers and poor prognosis with an increased risk of metastasis and death with high cathepsin D level

in tumors (from the Danish Breast Cancer Group, Copenhagen (13); the Centre René Huguenin, St-Cloud (14); W.L. McGuire laboratory, San Antonio (15)). This marker is independent of other classical prognostic markers including: lymph-node invasion, tumor size, Scarff and Bloom histological grade, Neu-*Erb*-B-2 and *Int*-2 oncogene amplifications, estrogen and progesterone receptors. It appears to be particularly useful in node-negative tumors in deciding for or against adjuvant therapy. The biological significance of the correlation between cathepsin D concentration and metastasis is not yet known. It could be the consequence of another process facilitating metastasis or more likely of one factor responsible for tumor cell invasion and metastasis, since cathepsin D is an overexpressed protease able to digest the extracellular matrix and several membrane proteins (16).

To conclude, cathepsin D appears to be a new important prognostic marker for breast cancer. This clinical application is the direct consequence of basic research at the molecular and cellular level aiming at understanding how estrogen works to stimulate human breast cancer growth and invasion.

This work has been supported by the "Institut National de la Santé et de la Recherche Médicale" and the University of Montpellier and monoclonal antibodies have been obtained in collaboration with SANOFI research laboratory.

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