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Cell Interactome: Good Neighbors or Bad Neighbors

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Abstract

We report an interesting finding that cellular distribution and interactions between neighboring cells modulate protein expression and localization. MCF7 breast cancer cells, which express ERb, were cultured in estrogen free medium. ERb exhibited a unique migration pattern that was dependent on cell density and proximity. This findings attest to a novel mechanism other than ligand-receptor one. Since cells were grown in conditioned culture media, the only factor influencing protein distribution is cell-cell proximity (cell-cell interactions). Thus, hormonal receptors may function independently from their ligand. in addition, physical intercellular interactions may function as a non-molecular factor inducing gene expression and activation.

Keywords

Estrogen; Estrogen receptor β ; gene expression; Receptor Translocation

INTRODUCTION

The central dogma in cancer progression focuses on monoclonal alteration of a single cell. The question arises: what triggers a good cell to turn into a bad one, and to what extent does the cellular neighborhood influence this alteration? Our fascinating observation here is that spatial distribution of cells, i.e. how far a cell is located from each neighboring one can modulate both levels of protein expression and its cyto-localization.

The spatial location of each cell, from among billions, is an important non-intramolecular variable in gene expression and protein localization, regulating organ development and integrity during both fetal development and in adult life. Yet spatially oriented cell signaling remains largely uncharacterized. Non-differentiated or cancerous cells lose their ability to act 'normally' in their designated locale, and develop altered signaling patterns. Just as contact inhibition and culture confluency alter cellular growth, loss of differentiation/orientation may presage cancer progression. Here, cells grown in estrogen-free media show varied localization and Estrogen receptor β (Er β) expression. Estrogen treatment induced receptor translocation

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to the cell membrane rather than to the nucleus, as one might expect, demonstrating that attachment and orientation are highly significant stimuli for modulation of protein expression and localization. This phenomenon was observed in both normal and cancer cells, yet was more pronounced in cancer cells.

There is longstanding debate over how the steroid hormones regulate target gene function [1]. On the one hand, estrogens bind receptors, activating and promoting gene expression, i.e. a 'genomic effect of estrogen.' Alternatively, estrogen may function by non-genomic mechanisms, through non-receptor modalities (MAP-kinase) [2]. Varied cellular response to hormonal therapy perplexes cancer researchers, who attribute this to cancer cell heterogeneity [3]. Yet, cellular interactions, cell density and their role in inducing hormonal response may be more complex. Cells within the same tissue type share structural phenotypes and receptor-ligand dynamics, and as shown here, may employ receptors as cell membrane proteins, to communicate with other cells.

HYPOTHESIS & DISCUSSION

It is well known that estrogen ligands with either its ER α or β receptor. The dynamic role of ER α is relatively well characterized, while that of ER β is less established [4]. Using immunohistochemistry, we have localized nuclear ER beneath the cell surface and found that cell density and cell-cell adhesion influence ER β 's sub-cellular localization. Our in-vitro model utilized the 'breast cancer cell' MCF7, well-known for studies on hormone receptor mechanisms. Two distinct cell groups were examined to understand the role of ER β in MCF7 cells in the absence of estrogen, i.e. a normal non-estrogen treated control group, and physiologic dosed (10^{-8} M) estrogen. The protein of interest (ER β) was labeled with red fluorescence, and Tritick conjugated Alexa-fluore secondary antibody served as the red channel.

In solitary cells (Fig. 1A), protein was uniformly distributed around the nucleus. Neighboring cells exhibited distinct sub-cellular protein localization. Protein became pronounced in areas of attachment indicating that ER β expression and localization are influenced by cellular locale. This is observed particularly when two adjacent or adherent cells exhibited protein patterns with characteristic polar tendencies oriented directionally along regions of cellular contact. With estrogen treatment, ER β translocated to the membrane, establishing a tethering junction between the cell membrane and nucleus (Fig. 1C). Relatively large amounts of protein also migrated toward cell protrusions and ruffled areas (Fig. 1D & 1E).

Without serum, estrogen, or phenol red in the cell media, the implication is that ER may respond to factors outside of estrogen alone. Boyan and co-workers found that treating ER expressing MCF7 cells with Estrogen and Estrogen-BSA, which cannot enter the cells, resulted in rapid increases in downstream PKC α gene expression [5]. Also, Tamoxifen, an estrogen agonist/antagonist, was shown to block PKC α expression but not by any ER antagonists (ICI-182780), ER-agonists (diethylstilbestrol), or ER antibodies. These results strengthen the notion of hormonal interaction with membrane pathways outside of receptor mediated interactions. An estrogen/membrane pathway interaction supports the role for estrogen non-receptor mediated pathways, via signal transduction prior to cellular entry [6] or in cytoplasm to nucleus translocation [7].

We demonstrate a relationship between cell density, cell-cell attachment, ER β localization and translocation. ER β appears to behave as a cell membrane protein in addition to conducting nuclear or mitochondrial [8] cellular transactions and may respond to non-estrogenic factors. This putative new role of ER β may apply not only to cancer cells, but also to neurons and other cell types.

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Figure 1. Representative Immunohistochemistry Images of ER β

ER β localizes in solitary cells around the nucleus (A, arrowhead). However, in cells that are in close contact or sub-confluent, ER β translocated to the area of interaction (B, arrowhead). Interestingly, following Estrogen treatment, the protein translocated from around the nucleus to the cell membrane, forming a tethering junction (C, arrowhead) and to areas of membrane protrusions (C1, arrowhead), ruffling, and pseudopodia (C2). [A, B, C $\times 1000$, C1 & C2 digitally magnified to $\times 10,000$].