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Integration of progesterone receptor mediated rapid signaling and nuclear actions in breast cancer cell models: Role of mitogen-activated protein kinases and cell cycle regulators

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Abstract

Progesterone receptor (PR) isoforms are dual functioning steroid hormone receptors, capable of activation of target gene transcription, and rapid stimulation of membrane-initiated intracellular signaling cascades. Herein we provided a retrospective of our recent work investigating the role of progestin-activated intracellular signaling pathways on cell cycle progression in breast cancer cell models. We show that progestin-induced S-phase entry and upregulation of selected target genes, including cyclin D1, are MAPK-dependent events. Further experiments conducted with mutant PRs defective in either the transcriptional response (PR-S294A) or activation of c-Src-dependent intracellular signaling to MAPKs (PR-mPro) confirmed that the proliferative response of breast cancer cells to progestins is largely dependent on the ability of PR to rapidly activate Erk 1/2 MAPKs. During progestin-stimulated cell cycle progression, elevated cdk2 levels and activity target multiple phosphorylation sites on PR. Phosphorylation of Ser400 augments PR nuclear localization and mediates increased PR transcriptional activity in the absence of hormone, while the cdk inhibitor, p27, reversed these effects. Together, our data illustrate the versatility of PR as regulatory signaling molecules that also act as sensors for multiple kinase pathways, and suggest that progestins influence changes in breast cancer cell gene expression and proliferation via integration of PR functions as both ligand-activated transcription factors and rapid initiators of intracellular signaling pathways.

Keywords: Progesterone receptor; MAP Kinase; Cyclin-dependent protein kinase; Cyclin D1; Breast cancer; Cell cycle progression

1. Introduction

Signal transduction is the process by which an extracellular stimulus is converted into an intracellular signal that triggers a cascade or series of events culminating in the production of an appropriate cellular response. The lipid bilayer is the primary barrier between a cell and its environment. Thus, external stimuli such as peptide growth factors must interact with transmembrane protein receptors to transmit their signal to the interior of the cell. Once bound by ligand, the receptor undergoes conformational changes that transduce the information to the cytosol, eliciting downstream steps of amplification, generation of second messengers, and/or integration with other signaling pathways. The signal is thus transformed and transmitted to intracellular target locations, such as the nucleus, to elicit a cellular response.

As an exception to this general scheme of signal transduction, the lipophilic nuclear hormone receptor ligands (including progesterone, estrogen, testosterone, etc.) can freely diffuse across the plasma membrane into the cytosol and nucleus, where they interact with their cognate protein receptors. Ligand binding triggers an "activating" receptor conformation change, dissociation of heat-shock protein chaperones, and receptor dimerization, ultimately leading to the recruitment of co-regulatory factors and direct alterations in the transcription of target genes. As such, steroid hormones have been considered unique in their ability to directly translate an extracellular signal into a nuclear response in the

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absence of intermediary proteins, generation of second messengers or activation of classical signal transduction pathways. This model of the primary function of members of the nuclear receptor (NR) superfamily as ligand-activated transcription factors that bypass intracellular signaling pathways has undergone recent revision. Instead, it now appears that rapid activation of MAPK and other cytoplasmic signaling pathways in response to steroid hormone binding is a common feature of NRs, and that roughly 5% of classically defined NRs are localized to the plasma membrane [1]. In breast cancer cells, progestins induce the interaction of a proline-rich region in the PR N-terminus with the SH3-domain of the soluble tyrosine kinase c-Src^{p60}, thus relieving inhibitory intramolecular interactions and catalyzing the downstream activation of the Ras/Raf/MEK/Erk MAPK cascade [2].

Activation of Erk 1/2 MAPK following exposure to progestins is a rapid and transient event (5–10 min), whereas the expression of PR target genes manifest on the order of hours to days. The physiologic significance of rapid intracellular signaling in response to treatment of breast cancer cells with progestins is unclear, but may serve to facilitate and/or "customize" several aspects of PR function, including the direction of PR subcellular localization, induction of PR target genes including integration with other hormones (i.e. transcriptional synergy), and modulation of PR protein turnover. Here we provide a summary of recent data produced in our laboratory and presented at the 2004 FASEB Summer Conference on Integration of Plasma Membrane and Nuclear-Initiated Signaling in Steroid Action. Our work suggests that progestin and PR-mediated rapid signaling events complement the nuclear actions of PR, but that PR stimulation of membrane-initiated signaling pathways can also regulate genes important for breast cancer cell growth independently of PR transcriptional or nuclear activities.

2. Experimental

2.1. Cell lines and reagents

T47D stable cell lines and culture conditions have been described previously [3]; MDA-MB-435 cells were grown in DMEM containing 10% FBS and 1% pen-strep. For experiments involving the synthetic progestin R5020 (10 nM; NEN, Boston MA), cells were plated in phenol-red free iMEM (Gibco) with 5% DCC serum. Inhibitors U0126 and PP2 (10 μM; Calbiochem) were added 30 min prior to addition of R5020. Phospho- and total-Erk1/2 MAPK antibodies were purchased from Cell Signaling; cyclin D1 antibodies were purchased from Upstate, and CDK 2 antibodies were purchased from Santa Cruz Biotechnology, Inc. Goat anti-mouse and goat anti-rabbit IgG horseradish peroxidase conjugate secondary antibodies were obtained from Bio-Rad Laboratories.

2.2. Immunoblotting and immunoprecipitation

Whole cell lysates were collected in RIPA buffer [4] followed by sonication for 10 s. For IP experiments, cells were collected in IP Buffer [5], sonicated twice for 10 s. Lysates were clarified by centrifugation for 10 min at 14,000 rpm at 4 °C. Soluble proteins were quantified by the Bradford method using Bio-Rad reagent, and equal amounts of protein were resolved by SDS-PAGE, electrotransferred to PVDF membrane (Millipore), immunoblotted with specific antibodies, and developed using SuperSignal® West Pico Chemiluminescent Substrate (Pierce) according to the manufacturer's protocol.

2.3. Transcription assays

T47D or MDA-MB-435 cells were transiently transfected using Effectene (Qiagen Inc.,) with various combinations of wt or S400A PR (10 ng each), cdk2 (1 μg), or p27 (1 μg), and a PRE-2x-TATA-luc reporter plasmid (10 ng) and pRL-tk (*Renilla* luc; 10 ng) plasmid as a control for transfection efficiency. Cells were treated as indicated and PR transcriptional activity in was measured using the Dual-Luciferase™ Reporter Assay (Promega) according to manufacturer's protocol.

2.4. Flow cytometry

T47D-YB, PR-null T47D-Y, or -S294A cells were plated in six well plates at a concentration of 100,000 cells/well, starved for 48 h, then treated accordingly. Cells were collected with trypsin, EtOH fixed, stained with propidium iodide, and analyzed as described [6].

3. Results and discussion

3.1. PR regulation of cell cycle target genes is Erk 1/2 MAPK-dependent

The function of PR isoforms as ligand-activated transcription factors has been well characterized, wherein hormone-bound PR dimers interact with specific DNA sequences, termed progesterone-response elements (PREs), located in the promoter or enhancer regions of target genes. PR-target genes that possess a canonical PRE include *c-myc* [7], *fatty acid synthetase* [8], and the *MMTV* promoter [9]. When bound to DNA, PR can function as a depot to localize chromatin remodeling activity by interacting with coactivator molecules, including the steroid receptor coactivator (SRC) family, and further enable the recruitment the general transcription machinery to galvanize the transcription process [10]. The promoter regions of PR target genes such as *p21* and *cyclin D1* do not encode a canonical PRE. Studies examining PR regulation of these genes have suggested a "tethering" mechanism, where, similar to the ER, PR can directly interact