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Review

Nuclear Phospholipase C Signaling Through Type 1 IGF Receptor and its Involvement in Cell Growth and Differentiation

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Abstract. *The existence of a nuclear polyphosphoinositol metabolism, independent from that at the plasma cell membrane, is now widely recognized. Specific changes in the nuclear phosphatidylinositol (PtdIns) metabolism have been implicated in cell growth, differentiation and neoplastic transformation. Here, the main features of nuclear inositol lipid signaling through type I IGF receptor, is reviewed with particular attention to the role of inositide-specific phospholipase C (PI-PLC) β1 in cell proliferation and differentiation, due to the peculiar localization of this molecule in the nuclear compartment.*

Nuclear phosphoinositide signaling

Polyphosphoinositides are important players in signal transduction, even though they are quantitatively minor constituents of cell membranes. It is known that phosphoinositide signaling involves the generation of the lipid second messengers, phosphatidylinositol 4-phosphate (PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2), in response to stimuli in a receptor-mediated manner at the plasma membrane. The existence of a nuclear polyphosphoinositol metabolism independent from that at the plasma cell membrane is now widely recognized (1). It has been demonstrated that nuclei contain many of the enzymes involved in the classic phosphatidylinositol (PtdIns) cycle, including kinases required for the synthesis of PtdIns(4,5)P2, PI-PLC and diacylglycerol kinase (DGK) (2-4). Specific changes in the nuclear PtdIns metabolism have been implicated in cell growth, differentiation and neoplastic transformation (5-7).

In this review, the major discoveries in the field of nuclear inositol lipid signaling through type I IGF receptor and, specifically, the role of nuclear PI-PLCβ1 in cell proliferation and differentiation are highlighted.

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IGF-I and Nuclear PI-PLCβ1 activation

An extensive body of evidence links PI-PLC to the nucleus (see ref. 3), but the main isoform in the nucleus is PI-PLCβ1. A possible activation of nuclear PI-PLC in quiescent Swiss 3T3 mouse fibroblasts mitogenically stimulated with insulin-like growth factor-1 (IGF-1) comes from a report showing that IGF-1 produced a decrease in PtdIns(4)P and PtdIns(4,5)P_2 mass and a concomitant increase in DAG levels, within 2 minutes of stimulation (8). Thus, activation of a nuclear PI-PLC seemed likely. No changes in the amounts of PtdIns(4)P, PtdIns(4,5)P_2, or DAG were detected in either whole cell homogenates or in nuclei in which the envelope was still present. Bombesin, another powerful mitogen for these cells, stimulated inositide metabolism at the plasma membrane level (as demonstrated by changes in the DAG mass measured in whole cell homogenates), but not in the nucleus. Concomitantly, our group has demonstrated the presence, in nuclei of Swiss 3T3 mouse fibroblasts, of PI-PLCβ1 the activity of which was up-regulated in response to IGF-1 stimulation (9).

Nuclear PI-PLCβ1 plays an important role as a mediator of the mitogenic stimulus exerted by IGF-1 on Swiss 3T3 mouse fibroblasts since inhibition of PI-PLCβ1 expression by antisense RNA renders these cells far less responsive to IGF-1, but not to platelet-derived growth factor (10). As a result of the increase in intranuclear DAG mass, PKC-α migrates to the nucleus (11).

How might PKC-α affect normal cell proliferation? Very recent findings have indicated that, in NIH 3T3 mouse fibroblasts treated with powerful tumor promoter 12-myristate 13-acetate (PMA), PKC-α and PKC-ε activate the cyclin D1 and cyclin E promoters and, thus, markedly elevate the levels of both cyclin D1 and E, resulting in higher proliferation rates. Increase of cyclin D1 expression is mainly mediated through the AP-1 transcription factor enhancer element present in the cyclin D1 promoter (12).

The regulation of nuclear PI-PLCβ1 upon IGF-I treatment

How nuclear PI-PLCβ1 is regulated is a key issue. The conventional view of PI-PLCβ1 activation comes from details of its action at the plasma membrane. It has been suggested that both Gαq/ α_{11} and Gβγ subunits can activate PI-PLCβ1. The region of PI-PLCβ1 that interacts with Gαq differs from that which interacts with Gβγ the former binding to the extensive C-terminal tail characteristic of the PI-PLCβ isoforms, while the latter has higher affinity for the N-terminal PH domain (16). There is yet no evidence that $\alpha q/\alpha_{11}$ is present in the nuclear compartment. Consistently, neither GTP-γ-S nor AlF4 stimulated PI-PLCβ1 *in vitro* activity in nuclei of MEL cells (17). A clue to a possible novel mechanism for the activation of nuclear PI-PLCβ1 has come from the observation that it is hyper-phosphorylated in Swiss 3T3 mouse fibroblast nuclei in response to IGF-1 and that this is abolished by preventing the translocation of p42/44 mitogen-activated protein kinase (MAPK) to the nucleus (18). Evidence was obtained with both insulin-treated NIH 3T3 mouse fibroblasts (7) and IL-2-treated human primary Natural Killer cells (19), where activation of nuclear PI-PLCβ1 was blocked by PD098059, an inhibitor selective for the MAP kinase pathway. Definitive proof of a direct involvement of MAPK has come from the demonstration that, following IGF-1 stimulation of quiescent Swiss 3T3 mouse fibroblasts, activated p42/44 MAPK translocates to the nucleus where it phosphorylates Ser 982 in the C-terminal tail of PI-PLCβ1 (20). This phosphorylation was inhibited by PD098059 and could be mimicked by recombinant PI-PLCβ1 protein and activated MAPK *in vitro*. This may represent an activation mechanism which is distinct from that at the plasma membrane and peculiar to the actions of the nuclear phosphoinositide cycle. In this regard, it may be significant that within the PI-PLCβ family, the β1 isoform is the only one which possesses a MAPK phosphorylation site in its C-terminal tail.

These are data suggesting that PI-PLCβ1 is deactivated by PKC-α and that this is a critical step in attenuating the phospholipase activity that drives the nuclear inositol lipid cycle (21). It would be interesting to know the effects of this point mutation of PKC-α on IGF-I-evoked mitogenesis.

The data presented in this review strengthen the contention for a direct link between the function of type 1 IGF receptor, translocation to the nucleus of activated MAPK and stimulation of nuclear PI-PLCβ1 signaling, which targets specific transcription factors involved in G1 progression and differentiation as well.

Nuclear phospholipase Cβ and myogenic differentiation

Nuclear PLCβ1 is a key player in myoblast differentiation, and functions as a positive regulator of this process. Differentiation of C2C12 myoblasts in response to insulin stimulation is characterized by a marked increase in nuclear PI-PLCβ1 (13). The timing of PI-PLCβ1 synthesis and its accumulation in the nucleus precedes that of the late muscle marker Troponin T by 24 hours and the expression of a transfected PI-PLCβ1 mutant lacking the nuclear localization signal acts as a dominant negative for nuclear translocation of PI-PLCβ1 and suppresses the differentiation of C2C12 myoblasts.

It has been proposed that myogenic factors regulate not only tissue-specific gene expression, but also the departure from the cell cycle. At the onset of differentiation, MyoD activates cyclin D3, which then sequesters unphosphorylated retinoblastoma protein, leading to the irreversible withdrawal of differentiating myoblasts from the cell cycle (14). This fits with our previous observations showing that a downstream target of nuclear PI-PLCβ1 signalling is the cyclin D3/cdk4 complex (15). A recent study (22) reveals that the C2C12 muscular cell line contains at least three PLCβ isoforms: $PLC\beta_1$, β_3 and β_4, while $PLC\beta_2$ was not detected. During myotube differentiation, in addition to large increases of both $PLC\beta_1$ forms in the nuclear compartment, there is also a marked decrease of $PLC\beta_4$ expression in the cytoplasmic and perinuclear compartment, while $PLC\beta_3$ expression does not change during the differentiation program. The different subcellular expressions of these PLC isoforms, namely the increase of nuclear $PLC\beta_1$ and the decrease of $PLC\beta_4$ during myotube differentiation, is related to a signalling event, IGF-1- or Insulin-dependent, involved in myogenic differentiation.

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