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Serum Deprivation Confers the MDA-MB-231 Breast Cancer Line with an EGFR/JAK3/PLD2 System That Maximizes Cancer Cell Invasion

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Abstract

Our laboratory has reported earlier that in leukocytes, phospholipase D2 (PLD2) is under control of Janus kinase 3 (JAK3), which mediates chemotaxis. Investigating JAK3 in cancer cells led to an important discovery as exponentially growing MDA-MB-231 human breast cancer cells, which are highly proliferative and metastatic, did not substantially use JAK3 to activate PLD2. However, in 2-h or 16-h starved cell cultures, JAK3 switches to a PLD2-enhancing role, consistent with the needs of those cells to enter a "survival state" that relies on an increase in PLD2 activity to withstand serum deprivation. Using a small-molecule tyrosine kinase inhibitor, the flavonoid 4',5,7-trihydroxyflavone (apigenin), as well as RNA silencing, we found that the invasive phenotype of MDA-MB-231 cells is mediated by PLD2 under direct regulation of both JAK3 and the tyrosine kinase, epidermal growth factor receptor (EGFR). Furthermore, serum-deprived cells in culture show an upregulated EGFR/JAK3/PLD2-PA system and are especially sensitive to a combination of JAK3 and PLD2 enzymatic activity inhibitors (30 nM apigenin and 300 nM 5-fluoro-2-indolyl des-chlorohalopemide (FIPI), respectively). Thus, a multi-layered activation of cell invasion by two kinases (EGFR and JAK3) and a phospholipase (PLD2) provides regulatory flexibility and maximizes the aggressively invasive power of MDA-MB-231 breast cancer cells. This is especially important in the absence of growth factors in serum, coincidental with migration of these cells to new locations.

Introduction

Neoplastic transformation and tumorigenesis have been associated with overexpression of PLD isozymes in cultured murine fibroblasts,[1] and high phospholipase D (PLD) activity has been documented in cancer cells.[2] Overexpression of either PLD1 or PLD2 results in the transformation of cells overexpressing a tyrosine kinase into a more malignant phenotype.[3] There is also a requirement for an intact PLD1 catalytic activity in H-RasV12-induced transformation.[4] PLD confers rapamycin resistance[5] and survival signals in human cancer cells with activated H-Ras or K-Ras.[6] PLD has been implicated, among other oncogenes, in colorectal,[7] renal,[8] and gastric cancers,[9] as well as melanoma.[10] PLD is possibly involved in metastasis and can induce *in vitro* tumor cell invasion,[11,12] while overexpression of PLD mediates matrix metalloproteinase secretion.[13] It has been recognized that PLD2 has a powerful effect on signal transduction, adhesion, migration, invasion, and metastasis in EL4 lymphoma cells.[14] The activation of this enzyme is found in lymphomas.[15] PLD also activates STAT3 that then activates the oncogenic kinase RET/PTC[14] and is able to form protein–protein complexes with the EGF receptor[16] (EGF-R) or with Pyk2 and Src kinases.[17]

The MDA-MB-231 human breast cancer cell line is highly proliferative and metastatic and was obtained at the MD Anderson Cancer Center.[18]

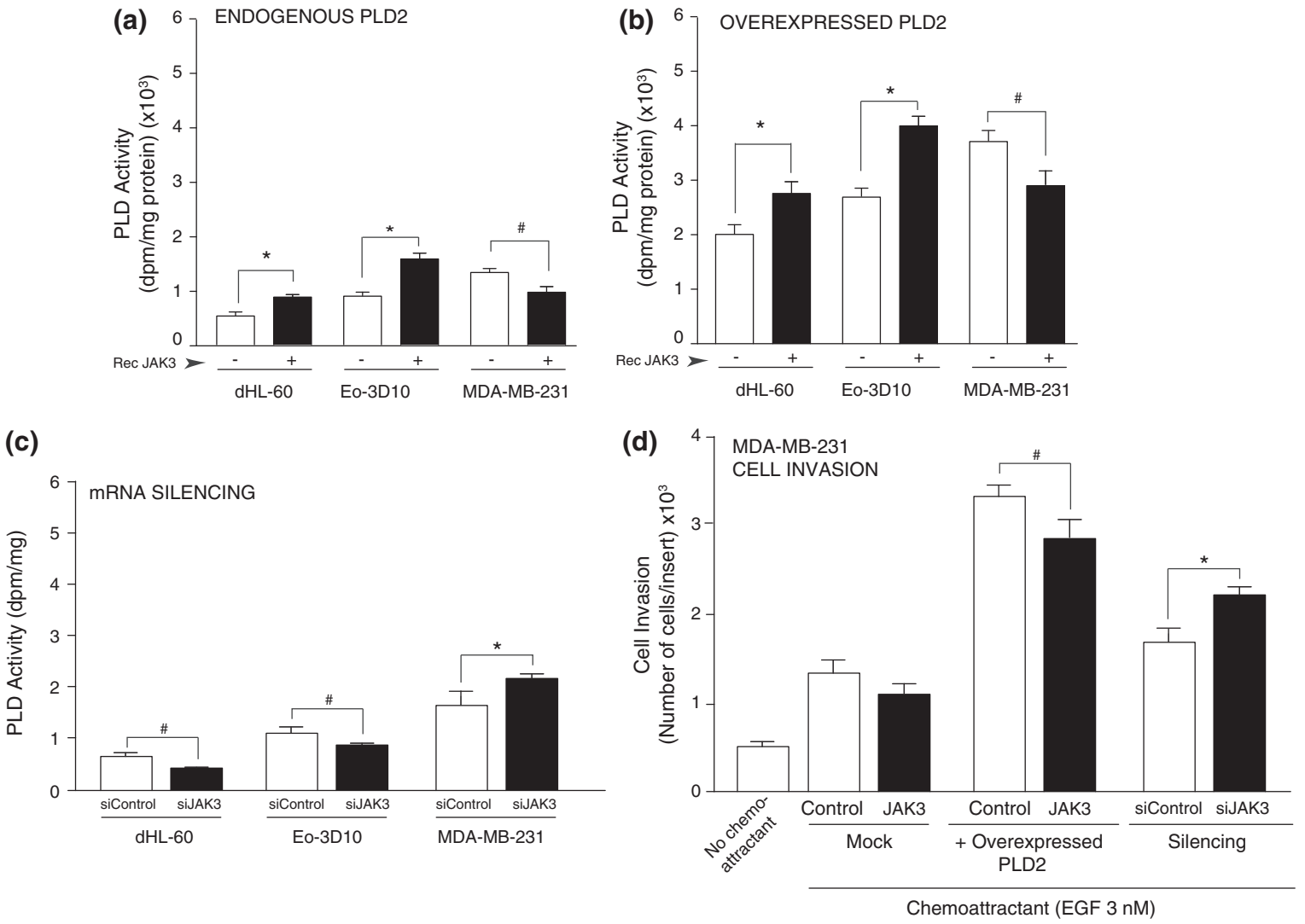

Fig. 1 (*legend on next page*)