

**Investigation of PI3K γ signaling
downstream of IGF-1R-CXCR4
transactivation in metastatic MDA-
MB-231 breast cancer cells**

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Abstract

Breast cancer metastasis is a multi-step process regulated by a number of homeostatic factors. The insulin-like growth factor 1 tyrosine kinase receptor (IGF-1R) and the chemokine G-protein coupled receptor, CXCR4 have been shown to play an important role in breast cancer metastasis. More recently, accumulating evidence suggest that these two distinct receptors may regulate breast cancer cell migration through receptor transactivation. However, the underlying molecular mechanisms by which IGF-1R-CXCR4 transactivation regulates breast cancer cell metastasis remain unclear. Since phosphoinositide 3 kinases (PI3Ks) are known to be key signaling molecules governing cell migration, PI3K signaling downstream of IGF-1R-CXCR4 transactivation was investigated. In the present study the expression of class I PI3K isoforms was investigated in metastatic MDA-MB-231 breast cancer cells compared to that in non-metastatic MCF-7 cells. The data show that high levels of class IB PI3K catalytic subunit, p110 γ are restricted to the highly metastatic cell types, correlating with the metastatic potential of the cell lines. Moreover, PI3K γ is the major PI3K isoform regulating cell migration and activation of Akt downstream of IGF-1R-CXCR4 transactivation in metastatic MDA-MB-231 cells. Finally, several downstream targets that are dependent on PI3K γ were identified using 2-D Fluorescence Difference Gel Electrophoresis (DIGE) and mass spectrometry analysis, including eukaryotic elongation factor 2 (eEF2), pyruvate kinase isozymes M1/M2 (PKM1/M2) and phosphoglycerate kinase 1 (PGK1) with PI3K γ being shown to regulate phosphorylation of eEF2. In summary, the data in this study demonstrate a novel role for PI3K γ in regulating cell migration downstream of IGF-1R-CXCR4 transactivation, potentially by attenuating cell proliferation via inhibition of eEF2 activation. The understanding of molecular mechanisms underlying receptor transactivation, including PI3K signaling transduction pathways in the progression of breast cancer metastasis and invasion may lead to development of more effective diagnostic and therapeutic strategies.

Declaration

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Abbreviations

Ab	antibody
BCA	bicinchoninic acid
bp	base pair
BSA	bovine serum albmin
°C	degrees Celsius
CCL	CC chemokine ligand
CCR	CC chemokine receptor
cDNA	complementary deoxyribonucleic acid
CXCL	CXC chemokine ligand
DIGE	Difference Gel Electrophoresis
DMEM	Dulbecco' s modifeied Eagle' s medium
DMSO	dimethyl sulfoxide
DNA	Deoxynucleic triphosphate
dNTPs	deoxynucleic triphosphates
eEF2	Eukaryotic elongation factor 2
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	estrogen receptor
FCS	fetal calf serum
g	gram
GPCR	G-protein coupled receptor
G protein	GTP-binding protein
GRK	G-protein coupled receptor kinase
GTP	guanine triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
HER2	human epidermal growth factor receptor 2
HRP	horseradish peroxidase
Ig	immunoglobulin
IGF-I or II	insulin-like groth factor I or II
IGF-1R	insulin-like groth factor-1 receptor

IGF-2R	insulin-like growth factor-2 receptor
IGFBP	insulin-like growth factor binding protein
IR	insulin receptor
IRS	insulin receptor substrate
JAK	Janus-family tyrosine kinase
kDa	kiloDalton
KIRA	kinase receptor activation assay
l	liter
MS	mass spectrometry
mTOR	mammalian target of rapamycin
m	metre
mA	milliampere
MAPK	mitogen-activated protein kinase
mg	milligram
ml	milliliter
mM	millimolar
mRNA	messenger RNA
μ	micron
μ g	microgram
μ l	microliter
n	nano
nm	nanometer
nM	nanomolar
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B
PKC	protein kinase C
PMSF	phenylmethylsulfonylfluoride

PTEN	phosphatase and tensin homolog deleted on chromosome ten
PTX	pertussis toxin
Raf	Ras activated factor
RNA	ribonucleic acid
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute medium
RTK	receptor tyrosine kinase
SDS	sodium dodecyl sulphate
Shc	src homology proteins
SOS	son of sevenless
S1P	sphingosine 1-phosphate
S1P1	sphingosine 1-phosphate receptor
STAT	signal transducer and activator of transcription
TAE	tris acetate EDTA
TBS	tris buffered saline
TEMED	N,N,N'-tetramethyl ethylenediamine
Tween-20	polyoxyethylene sorbitan monolaurate
V	volt
v/v	volume per volume
w/v	weight per volume