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PKB phosphorylates p27, impairs its nuclear import and opposes p27-mediated G1 arrest

JINHWA LEE[1], JIYONG LIANG[2] AND JOYCE M. SLINGERLAND[2]

[1]Department of Biotechnology, Dongseo University, Korea, [2]Sunnybrook and Womens College Health Sciences Centre, Toronto Sunnybrook Regional Cancer Centre 2075, Bayview Avenue, Toronto, Ontario M4N 3M5, Canada

Abstract

PKB activation may contribute to resistance to antiproliferative signals and breast cancer progression in part by impairing nuclear import and action of p27. PKB transfection caused cytoplasmic p27 accumulation and cytokine resistance. The nuclear localization region of p27 contains a PKB/Akt consensus site at threonine 157 and p27 phosphorylation by PKB impaired its nuclear import *in vitro*. PKB/Akt phosphorylated wild type p27 but not p27T157A. PKB activation led to cytoplasmic mislocalization of p27WT but p27T157A remained nuclear. In PKB activated cells, p27WT failed to cause G1 arrest, while the antiproliferative effect of p27T157A was not impaired. Cytoplasmic p27 was seen in 41% (52/128) of primary human breast cancers in association with PKB activation. Thus, we show a novel mechanism whereby PKB impairs p27 function that is associated with an aggressive phenotype in human breast cancer.

Introduction

p27Kip1 was first identified as a key mediator of TGF-1 induced G1 arrest Recent reports suggest that MAPK activation accelerates p27 proteolysis. The present study, taken together with those of the Viglietto and Arteaga groups,

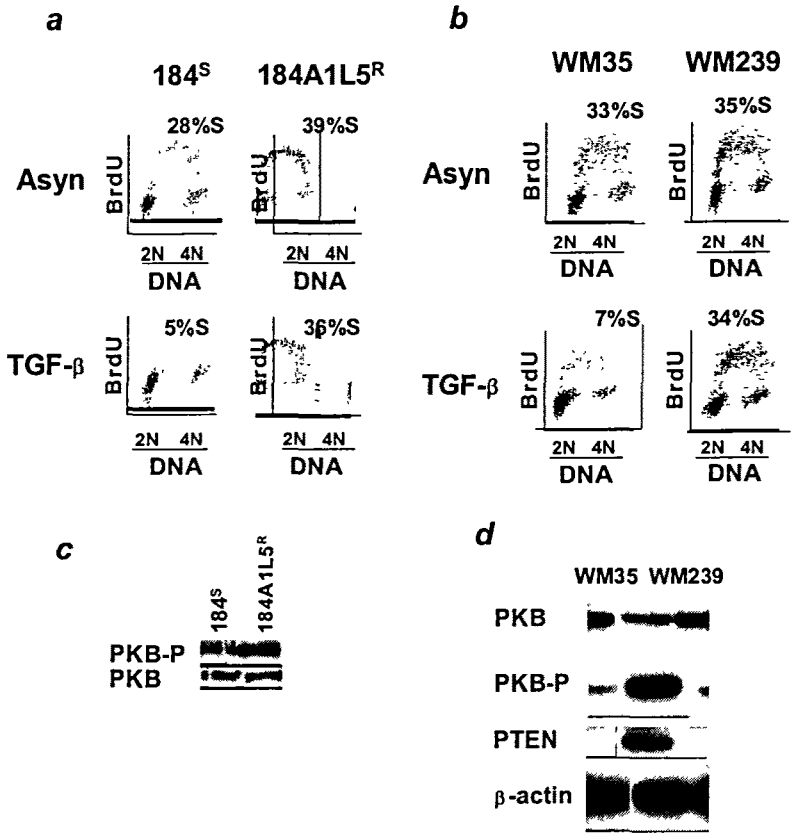

Fig. 1. Constitutive activation of PKB/Akt in TGF-b resistant cell lines. Proliferating 184A1L5R, 184, WM239 and WM35 were treated without (Asyn) or with TGF-b for 48 hrs. ***a*** and ***b*** show flow cytometric analysis in the presence or absence of TGF-b. ***c*** and ***d*** show immunoblots of activated PKB (PKB-P) and total PKB. PTEN and b-actin were also blotted (shown for the melanoma cell lines). While PKB phosphorylates a number of proteins involved in signal transduction, apoptosis, and gene expression, an increasing body of work suggests that this signaling pathway contributes importantly to cell cycle regulation. While this pathway affects multiple cell cycle effectors, including cyclin D and p21, the present study, together with those of Arteaga and Vigliettos groups define a novel mechanism linking PKB activation with impaired nuclear p27 import and p27 deregulation in human cancer.

suggest that activation of the PI3K/PKB pathway contributes to oncogenesis through inhibition of nuclear p27 import and hence its cdk inhibitory function. The demonstration that cytoplasmic mislocalization of p27 worsens the prognosis associated with reduced p27 levels alone and correlates with an aggressive tumor phenotype and poorer breast cancer survival supports the relevance of these mechanisms to human tumorigenesis. While some tumors show evidence of both accelerated p27 proteolysis and cytoplasmic localization, others show only one or the other. In human cancers, mutational activation of *Ras* and mutational loss of the tumor suppressor *PTEN* are not infrequent. Moreover, oncogenic activation of Ras by amplification or overexpression of receptor tyrosine kinases (RTKs), such as Her2/ErbB2, IGFR and EGFR can also activate PI3K/PKB in human breast and other cancers. Since both PI3K and MAPK are downstream of RTK/Ras, it will be of interest to determine what additional pathways direct RTK signaling to mediate either p27 proteolysis or cytoplasmic mislocalization in some breast cancers, while in others both co-exist. In certain tumors mislocalization of both p27 and p21 may have cooperative effects to promote tumor progression.

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