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Downregulation of gelsolin family proteins counteracts cancer cell invasion in vitro

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Abstract

Gelsolin and CapG are both actin binding proteins that modulate a variety of physiological processes by interacting differently with the actin cytoskeleton. Several studies suggest that overexpression of these proteins promotes invasion in vitro. In this study we explored the contribution of these proteins in human cancer cell invasion and motility. We show that down regulation of CapG or gelsolin in several types of cancer cells, including MDA-MB 231 and PC-3 cells, significantly reduces the invasive and motile properties of cells, as well as cell aggregation. These results point to a role for CapG and gelsolin as tumor activator.

Keywords: CapG; Gelsolin; siRNA; Invasion; Cell aggregation; Cytoskeleton; Cancer cell

1. Introduction

Regulation of cell motility, cell division, endo- and exocytosis and development embody just some of the traits of cells that are critically dependent on a dynamic actin cytoskeleton. In recent years, the role of actin and actin-associated proteins during tumorigenesis has been studied in more detail, most likely because of their connection with cell migration. Alterations in cell morphology and motility are important aspects of cancer cells, particularly during invasion and metastasis. However, in general it is has proven difficult to classify actin binding proteins either as a tumor activator or as a tumor suppressor. This apparent controversy is difficult to explain but may be related to the fact that investigators examine different types of cancer cells and

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cancer tissues. Alternatively, expression levels of these proteins may oscillate during cancerogenesis. While many proteins have been described to play a role in cell migration, the mechanisms through which they act remain unclear. Condeelis and coworkers [1] found that the expression of genes involved in motility (including a gelsolin-like actin filament capping-protein, cofilin, vinculin, zyxin, moesin) are dramatically upregulated in invasive tumor cells which predicts that cancer treatments targeting cell motility may be effective at killing these cancer cells. In the present study we focussed on two well-characterized actin-associated proteins, CapG and gelsolin, as targets for downregulation in human cancer cells.

CapG (gCap39) is a Ca^{2+}-sensitive actin binding protein that caps the barbed end of actin filaments, which results in polymerization inhibition of actin filaments [2,3]. Phosphatidylinositol 4,5-bisphosphate (PIP_2) counteracts capping activity [4]. Gelsolin, an F-actin filament severing and capping protein, is predominantly located in the cytoplasm whereas CapG localizes constitutively to both the nucleus and cytoplasm [5,6]. Nucleocytoplasmic shuttling of CapG does not occur in a passive manner but requires energy [7]. Neutrophils and bone marrow-derived dendritic cells isolated from CapG deficient mice show defects in motility [8]. Such motile functions are not affected in gelsolin-null macrophages, and no additional effects are observed in CapG/gelsolin-null macrophages suggesting that the function of CapG in macrophages does not interfere with the functions of gelsolin [9].

CapG is a target gene of AP-1, a transcription factor implicated in the regulation of tumor cell proliferation and Ras-dependent morphological transformation [10,11]. AP-1 is a dimeric transcription factor composed of members of the Jun and Fos proto-oncogene families [12]. When the aFos mutant, which causes inhibition of DNA binding after heterodimerisation with Jun, is expressed in an invasive fibrosarcoma cell line, a decrease in cell motility and repression of CapG expression are observed [13]. Microarray experiments have identified many differentially expressed genes that may contribute to enhanced invasion [1,14–16]. Using cDNA microarrays it was found that the CapG gene was highly expressed in pancreatic tumor tissues [17]. A comprehensive analysis of changes in gene expression in human glioblastomas, the most aggressive form of brain cancer, revealed that the CapG transcript was eightfold elevated [18], and CapG may also be associated with ocular melanoma [19]. Moreover, CapG, together with expression of immune response genes, was found to be significantly correlated with MMP-9 expression in lung adenocarcinomas [20]. We have previously shown that overexpression of CapG in non-invading cells promotes invasion of epithelial cells into a collagen type I matrix and into precultured chick heart fragments [7]. These events involve the Ras-PI3K pathway and CDC42 or RhoA, but not Rac1. Tagging CapG with a strong nuclear export sequence inhibited invasion, suggesting that nuclear and not cytoplasmic CapG elicits the invasive phenotype. On the other hand, Watari et al. [21] recently identified CapG as being downregulated in human cancer cell lines derived from a human diploid fibroblast lineage, suggesting that CapG can act as a tumor suppressor.

Gelsolin has been the subject of more intensive study in terms of its role in tumorigenesis [22–24]. While several reports indicate that gelsolin acts either as a tumor suppressor [25–28] or as a tumor activator [29–34], it may play a dual role in cancerogenesis. This is for instance substantiated by the findings of Rao et al. [35]. Decreased expression of gelsolin was found at early stages of malignant transformation in urothelial carcinomas. However, increased gelsolin expression was observed in the transition from non invasive to superficially invasive and to deeply invasive urothelial carcinomas, indicative of a biphasic expression pattern. They hypothesized that increased gelsolin expression may play a critical role in converting a superficial tumor to an invasive tumor. More recently, Shieh et al. [36] reported limited gelsolin staining in oral precancerous lesions, but increased gelsolin staining in primary and metastatic oral squamous cell carcinoma lesions, again reflecting a biphasic gelsolin expression pattern.

In the present work, we downregulated the expression levels of CapG and gelsolin in various human cancer cells using small interfering RNAs (siRNAs). Subsequently, the invasive and motile traits of these cells were analyzed. Our findings indicate that gelsolin and CapG are involved in the invasion cascade of cancer cells.

2. Experimental procedures

2.1. Cell culture

GF-PAC, BXPC-3, SU86.86, HeLa, MDA-MB 231 and HEK293T cells were maintained at 37 °C in a humidified 10% CO_2 incubator and grown in DMEM