Characterizing Gelsolin Expression in Colorectal Cancer

Lopez K.A.¹ and Yap C.T.²

Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore
Blk MD9, 2 Medical Drive, Singapore 117597

ABSTRACT

Gelsolin is a 90-kD actin-binding protein that mediates the severing and capping of actin filaments. This process is a vital step in a number of cellular events, including apoptosis and cell migration. Gelsolin is downregulated in the majority of tumor types, although it was also found at above-normal levels in certain cancers. In this study, two assays were performed in order to ascertain gelsolin’s possible roles and expression patterns in colorectal cancer. First, tissue samples were stained for the protein using immunohistochemical methods; second, two colorectal cancer cell lines were analyzed for gelsolin expression via immunofluorescence. It was observed that the majority of tissue samples displayed a consistent pattern of gelsolin downregulation in the tumor bulk and overexpression at the invasive or peripheral border, where tumor cells involved in invasion and metastasis are expected to be found. In the two cancer cell lines, gelsolin expression was localized to the membrane and cell periphery, particularly in cells that appear to be in the process of morphological change or locomotion. These findings provide supporting evidence that gelsolin may play a role in both programmed cell death, which is inhibited in cancer, and cell motility, which is a vital factor in a tumor’s invasive and metastatic potential.

INTRODUCTION

The gelsolin superfamily is responsible for controlling actin-based cytoskeletal dynamics. Gelsolin is involved in severing actin filaments, capping filament ends, and initiating filament synthesis by forming nuclei of actin monomers. Gelsolin acts as an actin-severing protein by catalyzing the non-proteolytic cleavage of filamentous actin.

Gelsolin has been found to be either overexpressed or downregulated in various tumor types. Overexpression was observed in pancreatic cancer cells, and subsequent knockdown led to a decrease in cell motility and, consequently, impaired metastasis (Thompson et al., 2006). In many other tumors, however, gelsolin levels are often undetectable or significantly lower than the norm. In non-small cell lung cancer tissues, cells with high gelsolin levels had decreased survivability relative to those with low gelsolin levels in patients with Stage II tumors, leading to the conclusion that high gelsolin expression has an adverse effect on NSCLC tumorigenesis (Yang et al., 2004). In total, around 60-90% of cancers display significant gelsolin underexpression, including those in the colon, stomach, and prostate (Kwiatkowski, 1999).

This study devotes a greater degree of attention on gelsolin’s potential role as a mediator of tumor invasion and metastasis. Actin severing by gelsolin is essential for the formation of structures such as pseudopodia, filopodia, and membrane ruffles that appear during cell crawling.

¹ Student
² Assistant Professor
In addition, one study revealed that gelsolin-null mice show impaired cellular translocation in fibroblasts and platelets, both of which normally have high baseline levels of gelsolin (Witke et al., 1995). Furthermore, inducing moderate increases in the gelsolin expression of NIH 3T3 mouse fibroblasts via gene transfection with human gelsolin cDNA resulted in improved rates of cell migration and wound healing in vitro that were proportional to the level of gelsolin in the cell (Cunningham et al., 1991). These observations and many others provide strong support for the hypothesis that gelsolin can be upregulated to enhance a tumor’s invasiveness and facilitate its metastasis.

MATERIALS AND METHODS

Two separate assays were performed to determine the presence, relative expression levels, and cellular localization of gelsolin in both normal colon cells and colorectal cancer cells. Immunohistochemistry (IHC) was done on three different sets of tissues – normal colon, primary tumor, and liver metastasis harvested from seven patients at the National University Hospital. The IHC protocol used involved horseradish peroxidase-antiperoxidase (PAP) and diaminobenzidene (DAB), resulting in a brown stain for gelsolin-positive cells. The chosen primary antibody dilution was 1:500. Hematoxylin was used to achieve a light violet nuclear stain.

The second assay performed was immunofluorescence (IF). Two different cell lines were grown and stained – HCT116 and E1. HCT116 is a cell line obtained from the cloning of human colorectal cancer cells. E1 cells are second-generation clones of HCT116 that were injected into the spleens of nude mice and subsequently metastasized to the mice’s livers. HCT116 has a characteristic epithelial morphology, while E1 is spindle-shaped. After some optimization, the chosen dilution for the primary antibody was 1:100. Two dilutions were used for the secondary antibody – 1:100 and 1:250. Hoechst 33342 was added after the secondary antibody in order to attain a blue nuclear stain.

RESULTS AND DISCUSSION

Gelsolin expression in normal colon, primary tumors, and liver metastasis

A scoring system was devised to rank the level of staining in selected parts of each tissue sample. For the normal colon, which was designated as the baseline from which the primary tumor and the liver metastasis would be graded, the chosen areas of interest were the mucosal surface, the crypts, and the interstitial space. The crypts display an overall negative staining for gelsolin, with a few isolated cells staining very highly. In contrast, the interstitial space has a generally high level of staining. In between these two is the mucosal surface, with an intermediate degree of staining.

The main bulk of the tumor has predominantly negative staining, similar to the level seen in the crypts, with highly stained pockets of cells near the center. One feature of interest is that the tumor bulk seems to be ringed by cells that have significantly darker staining than the tumor itself. However, since none of the patient samples showed a clear border between the tumor bulk and normal colon tissue, it cannot be concluded that the edges with highly stained cells form the invasive front of the tumor.

Similar to the main bulk in primary colorectal tumors, those in the liver metastasis have the negative level of staining observed in the crypts of the normal colon. Although the hepatocytes
are also seem to have little or no staining, they still appear to have a higher level of expression than the main tumor. Tumor cells between the main bulk and the normal liver are very strongly stained, surpassing even the level observed in the interstitial space of the normal colon. Furthermore, those highly stained cells display a very chaotic appearance, especially when compared to the distinctive structure of the main tumor and the ordered layers of the liver tissue.

Gelsolin in HCT116 and E1 cells
The fluorescent stain in both cell lines seems to be concentrated at the cell periphery, with decreasing intensity towards the nucleus. Prior to this study, Western Blot analysis performed by Dr. Tan Ee Hong has shown that HCT116 cells have intrinsically less gelsolin content compared to the more metastatic E1 cells, which is consistent with the results obtained from the 1:100 dilution. The E1 cells show particularly strong expression at the tips of their spindle shape and highlights at their edges, in contrast with HCT116 cells with their noticeable but relatively faint peripheral stain.

Gelsolin and apoptosis
Gelsolin’s role in mediating cytoskeletal remodeling, particularly of the actin cortex just beneath the plasma membrane, is involved in the characteristic morphological changes and cell blebbing that occur during apoptosis, the suppression of which is what allows most malignant tumors to form. The caspase-mediated cleavage of gelsolin allows it to mediate actin severing even in the absence of Ca\(^{2+}\), whereas uncleaved gelsolin can only perform its function with a certain concentration of intracellular Ca\(^{2+}\). Thus, it is not surprising that gelsolin seems to be significantly downregulated in the stained primary tumor and liver metastasis samples. However, uncleaved gelsolin seems to have a negative feedback effect on caspase-3 as an inhibitor of an upstream protein (Ohtsu et al., 1997) and as part of a complex that can directly inhibit caspase-3 itself (Azuma et al., 2000). Thus, gelsolin can exist as an anti-apoptotic protein that would be upregulated in cancer cells, which may explain the presence of some highly stained areas in the IHC analysis of both the main tumor bulk and the invasive border. This may also be related to the fluorescence observed in the cytoplasm of both HCT116 and E1 cells.

Gelsolin and cell motility
Gelsolin has been found to be essential in cell migration. The experiment done by Witke et al. clearly demonstrates that certain cells in gelsolin-null mice display impaired motility. In a similar study, the knockout of gelsolin in dermal fibroblasts led to the reduced formation of membrane ruffles necessary for cell crawling and caused the recombinant cells to move more slowly compared to their wild-type counterparts. When gelsolin was transfected back into the gelsolin-null cells, motility returned to normal. (Azuma et al., 1998) Conversely, inducing gelsolin overexpression in mouse fibroblasts resulted in a directly proportional increase in the rate of cell migration and wound healing in vitro (Cunningham et al., 1991). The requirement for severing proteins like gelsolin in cell locomotion is one probable reason for its upregulation in invading and metastatic tumors. Its apparent localization in tumor cells at the edges of the main tumor bulk supports this explanation, since these cells would be the most involved in translocating the tumor into surrounding tissues. The lack of organized structure in cells with high gelsolin expression may indicate that those parts of the tumor were in the process of invading or metastasizing when the tissue sample was harvested. The observed localization of gelsolin at the cell periphery of both HCT116 and E1 cells may also be related to gelsolin’s role...
in motility. The intense fluorescence at the cell edges may indicate recruitment of gelsolin to the parts of the membrane that would be involved in morphological changes and/or translocation. In light of these data and gelsolin’s involvement in cell motility, it seems very likely that the gelsolin upregulation via immunohistochemistry and the intense peripheral expression observed via immunofluorescence are indicative of gelsolin’s ability to enhance a tumor’s invasiveness and facilitate its metastasis.

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REFERENCES


