Intermediate filament Nestin and the cell motility in cancer – a review

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Abstract. – The intermediate filaments (IFs) constitute the cytoskeleton which is a key feature of both prokaryotic and eukaryotic cells. The IFs are expressed throughout life and are involved in the regulation of cell differentiation, homeostasis, ageing and pathogenesis. The IFs not only provide structural integrity to the cell, but they are involved also in a range of cellular functions from organelle trafficking and cell migration to signaling transduction. The IFs are highly dynamic proteins, able to respond and adapt their network rapidly in response to intra- and extra-cellular cues. In cancer, these IFs play a crucial role with regard to cell invasion via cell motility. The present review article will enlighten information about important IF Nestin with regard to its role in cancer cell motility and invasion.

Key Words: Nestin, Cancer, Cell motility, Invasion.

Introduction

The cytoskeleton consists of three distinct systems of protein polymers, all of which are engaged in essential functions for maintaining proper cell function, such as structural support, protein and organelle transport, cell shape, cell motility, mechanosensing, intra- and extracellular signaling. The least known of the cytoskeletal systems, the intermediate filaments (IF), also comprises the largest gene family coding for nearly 70 different proteins, each of which has distinct functions within the body. Within the IF family, there are two proteins of interest that are associated with cancer viz. nestin and vimentin.

Nestin is a rather unique entity in that it is expressed briefly, in very specific cell types, such as muscle, kidney and the central nervous system, at very specific times during a cells life – during development and regeneration. This very specific timing and location of expression suggest that nestin has a very specific function in allowing cells to transition through these processes. Nestin is also found to be expressed in a wide array of cancer types, yet until now its function in cancer has remained a mystery. The leading cause of death in cancers is metastasis that ultimately leads to the not formation of the secondary tumors. Metastasis occurs when cells in the primary tumor acquire the ability to invade and migrate, a term known as malignant transformation. The intrinsic changes a cell undergoes during this transition is commonly known as epithelial to mesenchymal transition (EMT). During this transition, tumor cells are able to break out from the primary tumor and invade into the surrounding tissue. Some cells will invade into the network of blood and lymph vessels that a tumor attracts to feed itself (intravasation). From there they can travel to many distal sites in the body, such as the lungs, liver and bones. To begin the reverse process of colonizing the tissue to which they have travelled, the cells need to exit the blood or lymph vessels (extravasation) into the stroma. The metastasized tumor cells will then undergo reverse EMT, also known as mesenchymal to epithelial transition (MET), whereby they lose their motile abilities and acquire some of the characteristics of the primary tumor. Nestin is most often expressed in intermediary stages of malignant transformation and is associated with cell migration and invasion. In the later sections, we will describe through understanding and details of nestin about cancer cell invasion and metastasis.

Intermediate Filaments Structural Characteristics

The IFs are very elastic in nature and are able to resist shear stress without breaking. Early studies suggested that one of the primary functions of the IFs was the maintenance of the basic structural
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Nestin in Differentiation and Angiogenesis

It is only in recent years that a knock-out (KO) mouse has been developed for nestin. The studies by Mohseni et al. and Yang et al. demonstrated that although nestin is not necessary for CNS development, it is important for peripheral motor function and development of the neuromuscular junction (NMJ). Nestin regulates acetylcholine receptor (AchR) clustering at the NMJ. Nestin is transiently expressed during myogenesis and is understood to regulate the pace at which myogenesis occurs by acting as an inhibitory scaffold for Cdk5, a myogenesis promoting kinase. Nestin clearly has a role to play in angiogenesis during regeneration and pathogenesis. It is also expressed in proliferating and metabolically active endothelium, independent of developmental and neoplastic processes.

The expression of nestin in angiogenesis may well be regulated by growth factors since mature endothelial cells cultured in the absence of growth factors had attenuated nestin expression. GFP is often coupled to a nestin regulatory element as a reporter to visualize angiogenesis during tumor progression. Nestin is expressed in the adult angiogenic vasculature following myocardial infarction, particularly in arteriovenous malformations. In the pituitary gland nestin is expressed during capillary neovascularization and is downregulated when pituitary infarcts transform to fibrotic tissue. Comparable with development, nestin expression in neovascularisation is transient and, as nestin is downregulated, vimentin expression is upregulated. While nestin appears to be expressed in the proliferating endothelium in the adult, it is unclear whether this is a result of increased proliferation or whether it confers specific functions to the newly formed endothelial cells and vasculature. Under shear flow conditions in the endothelium, nestin expression is decreased, which may reflect a need for cells to alter their proteome in order to resist this mechanical stress.

Nestin clearly has a role to play in angiogenesis by suppressing Cdk5, a myogenesis promoting kinase, in high glucose treated podocytes. Nestin can act downstream of caspase-3 to mediate apoptosis in high glucose treated podocytes. Nestin sequesters Cdk5 and regulates its activity by phosphorylation at T316. Nestin sequesters Cdk5 and regulate its activity by phosphorylation at T316. Nestin sequesters Cdk5 and regulate its activity by phosphorylation at T316. Nestin sequesters Cdk5 and regulate its activity by phosphorylation at T316. Nestin sequesters Cdk5 and regulate its activity by phosphorylation at T316.
is only released once a threshold has been reached that removes the inhibitory scaffold either by phosphorylation-dependent reorganization or through degradation. While the interactions with Cdk5 are the best characterized so far, other phosphorylation sites have been identified on nestin which will require further study.

**Nestin in Regeneration**

The fact that nestin expression rarely persists in fully repaired tissue lends itself to the notion that nestin plays a functional role in tissue repair. Nestin expression appears to be primarily in angiogenic structures and progenitor cells recruited to the regeneration site. During regeneration, nestin expression is induced by similar factors to those involved in differentiation, suggesting that regeneration could be used as a model to study nestin protein expression and function and vice versa. In myofibroblasts, nestin regulates DNA synthesis and proliferation which accelerates the healing process following ischemia. In the kidney, nestin has a slightly different function. In proximal tubule cells, nestin is transiently upregulated in response to hypoxia and TGFβ and regulates the migration of immature renal cells to the site requiring regeneration. Nestin is also upregulated by serum and PDGF in damaged mesangial cells, which surround the glomerulus. Nestin was shown to regulate their proliferation, but not their migration highlighting the cell-type specificity of nestin function. Nestin is also re-expressed in the pancreas, liver, skin, retina and teeth following trauma. However, its function in these regenerating tissues is poorly characterized.

**Nestin in Cancer**

Nestin has been identified in a number of cancers including osteosarcoma, prostate, breast, testicular cancer, ovarian, skin cancers, gastrointestinal tract cancers, lung cancer, pancreatic cancer, anaplastic thyroid carcinoma, angiosarcoma, glioma and other CNS tumors to name a few. During development nestin is considered as a progenitor cell marker, it is also a marker for cells in early neoplastic stages and during angiogenesis. The mechanisms that regulate nestin expression during development and regeneration may also regulate nestin expression during transformation. Several studies investigate the correlation of nestin expression in tumors with various clinical outcomes, such as prognosis, tumor grade, metastasis, recurrence and survival (Table I). In some cases, nestin expression did correlate with worse clinical outcomes, such as worse tumor grade or metastasis. However, this was not always associated with decreased patient survival. This variation could be due to study protocol differences, as well as a reflection of the potential complexity of nestin’s function in cancer. Much of the correlative data should be treated with care until there is a better understanding of how nestin functions in cancer.

### Table I. IF subtypes and associated diseases.

<table>
<thead>
<tr>
<th>Type</th>
<th>IF</th>
<th>Tissue</th>
<th>Disease</th>
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<tbody>
<tr>
<td>I and II</td>
<td>Keratins</td>
<td>Skin, Stratified epithelia, e.g. nails, hair</td>
<td>Pancreatitis, Liver disease, skin and hair-related tissue fragility disorders e.g. Epidermolysis Bullosa (EB)</td>
</tr>
<tr>
<td>III</td>
<td>Desmin</td>
<td>Striated and smooth muscle</td>
<td>Myopathies, Cardiomyopathy</td>
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<tr>
<td></td>
<td>Glial fibrillary acidic protein (GFAP)</td>
<td>CNS, peripheral nervous system (PNS)</td>
<td>Alexander Disease (AxD)</td>
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<td></td>
<td></td>
<td></td>
<td>Neurodegenerative diseases inc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alzheimer’s (AD), Parkinson’s (PD), Amyotrophic lateral sclerosis (ALS)</td>
</tr>
<tr>
<td>IV</td>
<td>Nestin</td>
<td>CNS, PNS, heart, kidney, muscle</td>
<td>Cancer and AD</td>
</tr>
<tr>
<td>V</td>
<td>Lamins</td>
<td>Nuclear lamina</td>
<td>Lipo and muscle -dystrophies, CMT, cardiomyopathies, Adult- onset autosomal dominant leukodystrophy (ADLD) and premature ageing diseases e.g. HGPS</td>
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In vivo nestin expression may come from the tumor and metastasis itself, but in other cases it appears to be a response by the surrounding tissue to the “injury” caused by the tumor\textsuperscript{30}. Nestin-expressing progenitor cells can be recruited to the tumor margin and to the tumor itself, either by the host or by the tumor secreted growth factors. The nestin-positive host cells recruited by tumors, such as gliomas, can both augment and inhibit functions such as tumor growth through angiogenesis and dissemination from the primary tumor\textsuperscript{31}. In other cases, the nestin expression comes from local upregulation in the tissue proximal to the tumor\textsuperscript{32}. This reflects nestin’s role in regeneration as opposed to a detrimental pathological role. It is critical in these cases to differentiate accurately between the cancer cells and the tumor microenvironment.

Conclusions

It is quite evident from the above literature that nestin plays a crucial role in cancer cell invasion and motility. The details provided shall help to further work on new drugs to target specifically various key attributes of this molecule to efficiently manage the critical process of cell invasion. This shall definitely allow efficient management of cancer cell metastasis, the real cause of mortality behind cancer.

Conflicts of interest

The authors declare no conflicts of interest.

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