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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 invasion vial cell motility. The present rev ticle will enlighten information about impor IF nestin with regard to its role in cancer cell m ty and invasion.

Key Words: Nestin, Cancer, C vasion.

Intro du

The cytoskelet nsists of t istinct systems of protei s, all of wh e engaged in essent unct r maintaining proper cell functi such as st l support, protein and org e transport, cel e, cell motility, mecha ensing intra- and e racellular signaling e least wn of the cytoskeletal systems, the filam (IF), also comprises the larg e fami ding for nearly 70 diffe- rotei h which has distinct functions wi the bo thin the IF family, there are two oteins of terest that are associated with c in and vimentin.

Nestin is a rather unique entity in that it is pressed briefly, in very specific cell types, such cle, kidney and the central nervous system, at specific times during a cells life – during development and regeneration[3,4]. This very specific timing and location of expression suggest that nestin has a specific fu allowing cells to tra ugh these p esses. Nestin is also fo to b essed in a wide array of cancer types, yet unti its function in cancer has d a mystery. eading cause of de- a cancers is metastasis that ultimately leads e not formati of the secondary tumors. Me- sis occurs v cells in the primary tumor a the abili o invade and migrate, a term kno t transformation. The intrinsic change undergoes during this transition is ommonly known as epithelial to mesenchymal n (EMT)[5]. During this transition, tumor ls 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 reacquire 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[6]. Early studies suggested that one of the primary functions of the IFs was the maintenance of the basic structural

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integrity of the cell. This elasticity and resistance to shear stress can be attributed to the hierarchical structure and specific structural changes during assembly of the IF filaments themselves[7]. IF proteins consist of an N-terminal "head" domain, a central rod and a C-terminal "tail" domain (Figure 1). IFs are assembled hierarchically. Dimers are formed through the interaction of two rod domains to form a coiled coil. The dimers assemble in an anti-parallel fashion to form tetramers which then assemble into unit length filaments (ULFs) composed of eight tetramers. The properties of IF filaments are not only conferred by their inherent structure and hierarchical assembly, the elastic properties of IFs are also regulated by ions. Support for the idea that the vimentin C-terminus is important for filament architecture come from a study showing that divalent cations, such as Ca^{2+} and Mg^{2+}, interacting with the last 11 amino acids on the vimentin tail promote stiffening of the filament network by crosslinking IF filaments[7].

Intermediate Filaments Contribute to a Diverse Range of Pathologies

The identification of IF functions in the [illegible] is often a result of their disease association. [illegible] the first identifications of intermediate filam[illegible] in disease were in cancer[9]. Common character[illegible] of many IF-related diseases arise from abnor[illegible] lities with filament assembly, fil[illegible] organiz[illegible] tion or aberrant regulation ([illegible] degrada[illegible] tion), all of which can hav[illegible] ownstr[illegible] effects on protein-protein intera[illegible] and c[illegible] ling. In some cases, the IF i[illegible] qu[illegible] preg[illegible], other times, as with [illegible] chinso[illegible] ord Progeria Syndrome (HGPS) [illegible] the keratin[illegible] blistering diseases, the p[illegible] ises from [illegible] on-altering mutatio[illegible] many [illegible] rders associated with type 4 IFs [illegible] characteri[illegible] abnormal protein aggregat[illegible] arising from mi[illegible] lation of PTMs and e[illegible] ed pr[illegible] in expressio[illegible]. Understanding the [illegible] the IF[illegible] y in disease has facilitated the unde[illegible] di[illegible] these [illegible] teins' function during homeos[illegible]

Ne[illegible] in D[illegible] ntiation and An[illegible] genesis

[illegible] ecent years that a knock-out (KO) [illegible] use has been developed for nestin. The studies [illegible] Mohseni et al[10] and Yang et al[11] demonstrated [illegible] lthough 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 inhibi[illegible] fold for Cdk5, a myogenesis promot[illegible] kinase. Nestin clearly has a role to play i[illegible] giogenesis during regeneration and pathoge[illegible] It is also expressed in proliferating and metab[illegible] ly active endothelium, independ[illegible] of deve[illegible] ntal and neoplastic processes[12].

The expression of n[illegible] in an[illegible] genesis [illegible] well be regulated by [illegible] th fa[illegible] s since nature endothelial cells c[illegible] re[illegible] sence [illegible] owth factors had atte[illegible] ed nest[illegible] ress[illegible] GFP is often couple[illegible] nestin regu[illegible] ment as a reporter t[illegible] angiogenes[illegible] uring tumor progressi[illegible]. Nest[illegible] xpressed in the adult angiogenic vasculature [illegible] ing myocardial infarct[illegible] arly in arteri[illegible] us malformations[16]. I[illegible] pituitary gland nestin is expressed during [illegible] llary neova[illegible] arization and is downregulated when pituita[illegible] nfarcts transform to fibrotic t[illegible][17]. Compa[illegible] e with development, nestin exp[illegible] ascularisation is transient and, as nesti[illegible] wnregulated, vimentin expression is upregulated. While nestin appears to be expressed in the proliferating endothelium in the adult, it [illegible] r whether this is a result of increased proliferation or whether it confers specific functions to the newly formed endothelial cells and vasculature[18]. 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[19].

Scaffolding and Cytoprotective Functions of Nestin

Nestin has a cytoprotective function in both neurons and podocytes in the kidney, and this is related to its interaction with and reciprocal regulation of Cdk5. Cdk5 regulates nestin filament organization[20] by phosphorylation at T316. Nestin sequesters Cdk5 and regulate its activity by modulating the Cdk5 activators p35/p25. During stress, such as oxidative stress or high glucose situations, nestin is degraded resulting in sensitization of the cells to Cdk5 pro-apoptotic activity[21]. Under stress conditions, Cdk5 is upregulated and acts upstream of caspase-3 to mediate apoptosis in high glucose treated podocytes. Nestin can attenuate this effect[22] presumably by sequestering Cdk5. In vascular smooth muscle cells oxidative stress leads to nestin upregulation which inhibits apoptosis by Cdk5 sequestration[23]. Cdk5

Table I. IF subtypes and associated diseases.

Type	IF	Tissue	Disease
I and II	Keratins	Skin, Stratified epithelia, e.g. nails, hair	Pancreatitis, Liver disease, sk and hair-related tissue fragi disorders e.g. Epidermol Bullosa (EB)
III	Desmin	Striated and smooth muscle	Myopathies, Card opathy
	Glial fibrillary acidic protein (GFAP)	CNS, peripheral nervous system (PNS)	Alexander D (AxD) Neurodege ive dise nc. Alzheim). Pa on's (PD). vot al sclero LS)
	Peripherin	CNS, peripheral nervous system (PNS)	A notor neuron egeneration
IV	Nestin	CNS, PNS, heart, kidney, muscle	Cancer D
V	Lamins	Nuclear lamina	Lipo and mus strophies, CMT, cardiomyopathic Adult- onset autosomal dominant leukodystrophy (ADLD) and pre e ageing diseases e.g. HGPS

is only released once a threshold has been reached that removes the inhibitory scaffold r by phosphorylation-dependent reorganiz through degradation. While the interaction th Cdk5 are the best characterized so far, other sphorylation sites have been identified on nes which will require further study[24].

Nestin in Regeneration

The fact that nestin ex on rare in fully repaired tissue le ds n ot nestin plays a funct role e repair. Nestin expression ar s to be pri in angiogenic structure enitor cell uited to the regenerat ite. g regeneration, nestin expression induced by ar factors to those involved ifferentiation, sting that regenerati ould b sed as a mo el to study nestin pro expres and function and vice versa. In myo la nestin ulates DNA synthesis and pr ion wh accelerates the healing ss fo g emia[25]. In the kidney, nesti a slig ferent function. In proximal tub cells, nes n is transiently upregulated in r oxia and TGFβ and regulates the gration of immature renal cells to the site requiring regeneration[26]. Nestin is also upregulated um and PDGF in damaged mesangial cells, ich surround the glomerulus. Nestin was shown to regulate their proliferation, but not their migration highlighting the cell-type specificity of nestin is also re-expressed in the pancrea , skin, retina and teeth following rauma. However, its function in these regenera- es 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[27]. 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[28]. 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[29]. 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.

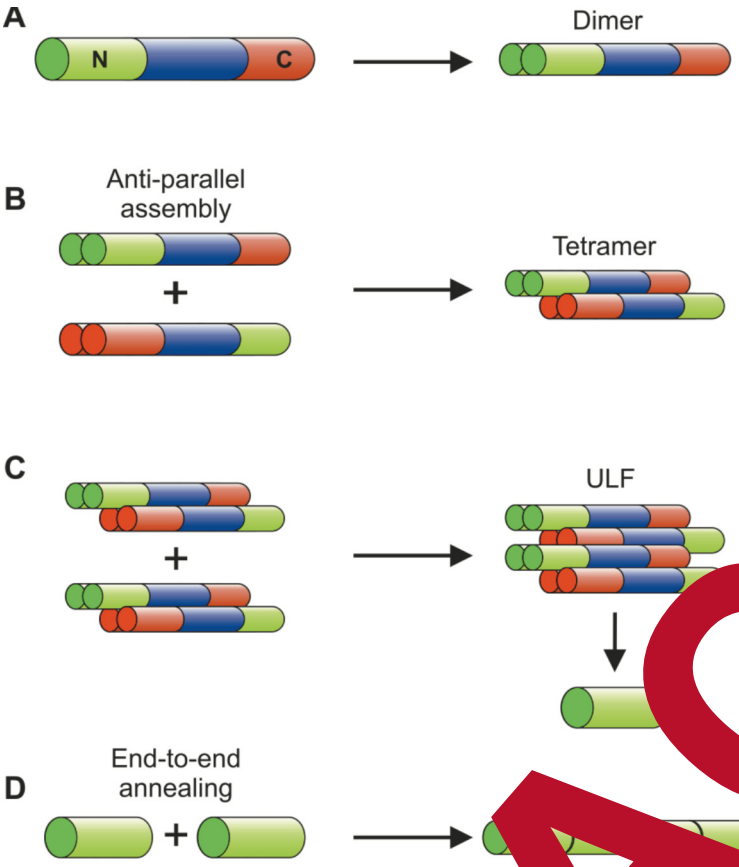

Figure 1. Intermediate filaments.

In vivo nestin expression may [illegible] from the tumor and metastasis itself, but it appears to be a response by the surrounding tissue to the "injury" caused by the tumor. Nestin-expressing progenitor cells can be recruited to the tumor margin and to the tumor itself, either from the host or by the tumor secreted growth factors. Also, nestin-positive host cells recruited by tumors, such as gliomas, can both augment and inhibit functions such as tumor growth through neovascularization and dissemination from the primary tumor. In other cases, the nestin expression comes from local upregulation in tissue proximal to the tumor[32]. This reflects nestin role in regeneration as opposed to a detrimental pathological response is critical in these cases for differential accessibility 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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