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Vimentin and post-translational modifications in cell motility during cancer – a review

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Abstract. – The post-translational modifications (PTMs) are defined as the covalent modification or enzymatic modification of proteins during or after protein biosynthesis. Proteins are synthesized by ribosomes translating mRNA into polypeptide chains, which may then undergo PTM to form the mature protein product. PTMs are important components in cell signaling. Moreover, it is a known fact that PTM regulation offers an immense array and depth of regulatory possibilities. The present review article will focus on their possible role in cancer cell motility with special reference to vimentin, an intermediate filament (IF), as the later is an important process responsible for life-threatening state viz. cancer metastasis.

Key Words: Post-translational modifications, Intermediate filaments, Cancer, Metastasis, Vimentin.

Introduction

Intermediate filaments (IFs) are regulated at transcriptional, translational and post-translational levels[1]. The prime focus of the present report is on the regulation of IFs either by their posttranslational modifications (PTM) or by their assembly. PTMs are one of the key regulators of IF function. The combination of allosteric and orthosteric PTM regulation combined with the fact that many PTMs cross-talk, provides an array of regulatory possibilities. This has been referred to as the "PTM code" that actually infers how proteins regulate a diverse range of biological functions[2].

The PTM namely eukaryotic phosphorylation involves the addition of a phosphate group to a serine (S), threonine (T) or tyrosine (Y) residues on a protein. It is catalyzed by a kinase, while protein phosphatases catalyze the removal of the phosphate group. Of note, kinases make up around 2% of the genome[3], whereas there are approximately 50% phosphatases which in turn implies that phosphatases are less specific than kinases[4]. Sequential protein phosphorylation events can act as a form of signal amplification. Co-operative phosphorylation events require several phosphosites that are usually modified before a signal is generated. Each of these types of multi-phosphorylation events can be considered as a threshold to regulate filament organization or cell signaling events. While much of the focus on the next couple of sections is on the action of phosphorylation and kinases, dephosphorylation by phosphatases is also equally important to maintain phosphoprotein homeostasis since hyperphosphorylation of IFs is a trigger for many diseases including cancer.

Overview of Intermediate Filament Phosphorylation

IF phosphorylation as a regulator of IF organization and function is implicated in a whole host of cellular processes and pathologies[5]. Phosphorylation of IFs typically occurs on the head and tail domains. Phosphosites have been predicted by mass spectrometry (MS) on the central helical rod domains, although these are unvalidated[6]. Phosphorylation is a transient PTM that regulates the dynamic assembly and disassembly of IF filaments[7]. Often the protein aggregation is a misregulation of phosphorylation processes, which can result in the diseased state. As such, control of phosphorylation needs to be tightly regulated to preserve cellular and tissue homeostasis. The roles of phosphorylation in IF regulation are three-fold viz. IF assembly, protein-protein inter-

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actions and signaling; however, they are all interconnected. Phosphorylation on the vimentin head domains disrupts IF dimerization[8] and it reduces the affinity of IF head domains for the central rod domains, thus promoting disassembly[9]. The influence of phosphorylation is more complex, as it can also induce structural changes in the non-adjacent linker 2 and C-terminus[10]. In the context of the discussion above, this type of phosphorylation event would be considered allosteric. It must be stressed that most of these structural studies have been conducted in *in vitro* systems and may not mimic the reality of *in vivo* vimentin assembly/disassembly. Examples of the other roles of phosphorylation as a regulator of protein-protein interactions and signaling will be covered in the next section with a particular focus on vimentin.

Vimentin Phosphorylation

Vimentin has 23 identified phosphorylation sites on its N-terminus and C-terminus[11]. The N-terminus phosphosites are primarily regarded to regulate vimentin organization and protein-protein interactions. In particular, it seems that certain phosphosites are specifically phosphorylated by one or two kinases. On the other hand phosphosite such as S38, observed to be commonly phosphorylated by a large set of kinases, leading to a broad array of functions. Besides emerging research on IF phosphorylation functions and interactions, we are still very much in the discovery phase of finding out what each site does and to what physiological processes it contributes. In the future, it will be interesting to consider the added diversity brought by cell type-specific phosphorylation functions. For instance, nestin exhibits differential phosphorylation according to cell type, with some nestin phosphosites being CNS-specific[12]. This could be another explanation as to why vimentin C-terminus functions have remained elusive – they simply have not been investigated under the right conditions. The majority of the validated phosphosites on vimentin are serines. There are also five tyrosine phosphorylation sites on the vimentin head-domain that have been identified by several independent studies, but none has been validated *in vivo*[6]. As such all of the discussion on the phospho-regulation of vimentin is concentrated on serine phosphorylation.

Vimentin Phosphorylation and Cell Migration

Vimentin promotes cell migration which is a co-ordinated process involving the formation of the leading edge, lamellipodia extension, adhesion, locomotion of the cell body and retraction of the trailing edge. Activation of two key effectors of migration, cell division control protein 42 homolog (Cdc42) and Ras-related C3 botulinum toxin substrate 1 (Rac1) causes tyrosine phosphorylation-dependent collapse of the vimentin network[13]. Furthermore, nothing is known about the specific sites of vimentin tyrosine phosphorylation; it has merely been reported to exist in a few studies, several of which connect it to the activity of the migration effector PI3K[14]. PI3Kγ-dependent phosphorylation of vimentin on serines namely S6 and S38, induces vimentin filament reassembly and retraction from the cell periphery. In particular, inhibition of the phosphorylation of S6 and the adjacent serines impaired transendothelial migration, demonstrating that disassembly of the vimentin network is important for proper cell motility. Since PI3K does not directly phosphorylate vimentin, it could act upstream through several effectors to induce vimentin phosphorylation, such as Rac, or Akt. Akt1 is a known scaffolding target of vimentin; it phosphorylates vimentin at S38, and facilitates cell migration. Inhibition of PI3K prevented the Akt1 phosphorylation of vimentin thereby, confirming PI3K kinase as an upstream activator of Akt induced vimentin phosphorylation[15]. While the effect of S38 phosphorylation on the vimentin network was not shown, it can be assumed that vimentin assembly was disrupted thus enabling proper migration and tumorigenesis.

There is a gradient of vimentin organization in motile cells, from primarily non-filamentous precursors in the distal regions of lamellipodia, with vimentin gradually becoming more organized further away from the lamellipodial edge towards the nucleus, suggesting that vimentin is dynamically organized during cell motility[16]. A cluster of serines at the N-terminus of vimentin, including S6, is phosphorylated in a PKCε-dependent manner which is important for integrin recycling[17]. During cell spreading, vimentin is phosphorylated in a PKCε dependent manner at S6, S38, and S50. Filamin A, an actin cross-linker, forms a complex with activated PKCε and phosphorylated vimentin, and this is required for vimentin reorganization in spreading cells[18]. This has been further verified by studies showing that an organized vimentin network is antagonistic for lamellipodia formation, but is necessary for establishing cell polarity. Rac induced vimentin phosphorylation at S38 induces network disassembly and retraction from the lamellipodia pri-

or to membrane ruffling[16]. Notably, inhibition of ROCK, a known vimentin kinase, and Rac, an upstream activator of another vimentin kinase PAK, has differing effects on vimentin solubility depending on whether cells were grown on soft or stiff substrates[19]. These results are pertinent considering that malignant tissues exhibit increased ECM stiffness; as such vimentin turnover could be affected in malignant situations, potentially altering migratory abilities. It is not just the presence of vimentin that is important, but also its assembly status, as vimentin disassembly act as a molecular clutch which interacts as well as modulates the actin machinery during cell migration. On the other hand, assembled vimentin acts as a brake[16]. Further, It is now a known fact that the vimentin phosphorylation is important for the recycling of integrins that are essential for adhesion disassembly[17].

Role of Vimentin Dephosphorylation

Rearrangement of the vimentin cytoskeleton in a reversible phospho-dependent manner requires the co-ordination of kinases and phosphatases. Despite the wealth of research describing the importance of vimentin phosphorylation in vimentin assembly and function, the remarkably little effort has been invested into understanding the role of dephosphorylation. Inhibition of dephosphorylation causes hyperphosphorylation and disassembly of IFs, suggesting that phosphorylation and dephosphorylation act in equilibrium to maintain filament organization[20]. Protein phosphatase 2A (PP2A) is one of the first identified vimentin phosphatases which interacts directly with vimentin and plays an important role in filament reassembly[21]. Moreover, intracellular Ca^{2+} is also important for vimentin dephosphorylation and is most likely through Ca^{2+} activation of protein phosphatases.

Other PTM's Role in the Regulation of Intermediate Filament

While phosphorylation has been the primary PTM regulator of IF dynamics studied over the years, other PTMs are starting to get a look in. What has been emerging in recent years is that there is also a cross-talk between PTM types, although this aspect of IF regulation is still very much in its infancy.

Intermediate Filaments are Sumoylation and Scetylation Targets

Keratins are sumoylated and the degree of sumoylation regulates keratin solubility. Keratin sumoylation is associated with stress-induced phosphorylation and ubiquitination, although exactly mechanisms remain to be studied[22]. In the same report, it was shown that vimentin is SUMOylated. Although the functional significance of vimentin SUMOylation is currently unknown, it is not unreasonable to assume that it might interact with phosphorylation to regulate vimentin organization. Keratins are also acetylated, which can be up-regulated in response to glucose stimulation. Interestingly, K8 acetylation directly modulates K8 phosphorylation which has consequences for K8 filament organization and solubility[23].

Phosphorylation and Glycosylation Cross-Talk

A more established example of IF PTM cross-talk is that of glycosylation and phosphorylation. Glycosylation involves the addition of an oligosaccharide, or a single sugar molecule, to asparagine (*N*-linked), or serine and threonine (*O*-linked) residues. Unlike acetylation and sumoylation, which occur on lysines, *O*-GlcNAcylation commonly occurs on the same, or adjacent to phosphorylatable serine/threonine residues. Thus, glycosylation can compete with phosphorylation for the same residues. It can also stimulate phosphorylation and vice versa. This can give phosphorylation and glycosylation complementary and competing functions known as the 'yinyang' hypothesis[24].

Conclusions

Firstly, protein responsible for cancer cell motility plays an important role in the crucial process of metastasis. Furthermore, these are post-translational modifications which are power regulators for these motility-inducing proteins. So, further researches are required to get in-depth mechanisms working collectively to affect cell motility. This will result in the better understanding of targeted therapeutics for cancer metastasis.

Conflicts of interest

The authors declare no conflicts of interest.

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