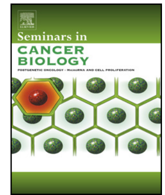
Seminars in Cancer Biology 27 (2014) 39–45

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer



Review

Snail nuclear transport: The gateways regulating epithelial-to-mesenchymal transition?

Irfana Muqbil[a], Jack Wu[a], Amro Aboukameel[a], Ramzi M. Mohammad[a,b], Asfar S. Azmi[c,*]

[a] Department of Oncology, Karmanos Cancer Institute, USA
[b] Division of Research, Hamad Medical Corporation, Doha, Qatar
[c] Department of Pathology, Wayne State University, School of Medicine, Detroit, MI, USA

ARTICLE INFO

Keywords:
EMT
Nuclear transport
Karyopherin
Xpo1
CRM1

ABSTRACT

Epithelial-to-mesenchymal transition (EMT) and the reverse process (MET) play central role in organ developmental biology. It is a fine tuned process that when disturbed leads to pathological conditions especially cancers with aggressive and metastatic behavior. Snail is an oncogene that has been well established to be a promoter of EMT through direct repression of epithelial morphology promoter E-cadherin. It can function in the nucleus, in the cytosol and as discovered recently, extracellularly through secretory vesicular structures. The intracellular transport of snail has for long been shown to be regulated by the nuclear pore complex. One of the Karyopherins, importin alpha, mediates snail import, while exportin 1 (Xpo1) also known as chromosome maintenance region 1 (CRM1) is its major nuclear exporter. A number of additional biological regulators are emerging that directly modulate Snail stability by altering its subcellular localization. These observations indicate that targeting the nuclear transport machinery could be an important and as of yet, unexplored avenue for therapeutic intervention against the EMT processes in cancer. In parallel, a number of novel agents that disrupt nuclear transport have recently been discovered and are being explored for their anti-cancer effects in the early clinical settings. Through this review we provide insights on the mechanisms regulating snail subcellular localization and how this impacts EMT. We discuss strategies on how the nuclear transport function can be harnessed to rein in EMT through modulation of snail signaling.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Epithelial-to-mesenchymal transition (EMT) is a complex phenomenon in which cancer cells lose their polarity undergo changes from epithelial to mesenchymal morphology thereby achieving plasticity that confers an invasive and metastatic behavior [1]. It is a fine tuned process regulated by a number of proteins that are strategically distributed in the nuclear and cytosolic compartments of cancer cells. A number of different parallel signaling pathways interact in the development of EMT [2]. Over the years, these pathways have been well studied leading to deeper characterization of different EMT promoting proteins and transcription factors (TFs), as well as their localization within and outside the cellular compartments [3]. Major EMT regulating proteins and TFs, such as wnt/β-catenin, notch, TGF-β, Twist and Snail are recognized to undergo nuclear-cytosolic shuttling using specialized transporters: karyopherins [4]. These observations highlight that the nuclear protein transport process may be playing an integral part in the EMT signaling. The karyopherin importin alpha shuttles in proteins with nuclear localization signal sequence (NLS) [5]. The export of most of the EMT regulating TFs is mediated exclusively by Exportin1/XPO1 [also known as chromosome maintenance region 1 (CRM1)] through nuclear exclusion sequence (NES) recognition [6]. For long it has been fairly well recognized that in addition to transcriptional regulation, the activity of the TFs can be controlled by changing their cellular location which permits rapid response to signals, resulting in a powerful modulation of the biological system [7]. While, disease induced changes in expression of nuclear importer proteins have not been very well characterized, it is fairly unequivocally well recognized that nuclear exporters particularly CRM1, are often aberrantly expressed in cancer [8]. Nevertheless, till date, not many studies have looked into how disturbed nuclear export may interfere with EMT signaling. As discussed below, we propose that studying the role of aberrant nuclear export is critical

* Corresponding author at: Department of Pathology, Wayne State University, School of Medicine 4100 John R, HWCRC 732, Detroit, MI 48201, USA.
Tel.: +1 3135768328; fax: +1 3135768389.
E-mail addresses: azmia@karmanos.org, dt9860@wayne.edu (A.S. Azmi).

http://dx.doi.org/10.1016/j.semcancer.2014.06.003
1044-579X/© 2014 Elsevier Ltd. All rights reserved.

to understanding the regulation of EMT through major TFs that they directly target (here a special focus on the Snail protein). As a number of different specific inhibitors of nuclear export are being developed and tested clinically, this may become an attractive therapeutic strategy to interfere in the EMT pathways.

2. The Snail family transcription factors and EMT

The transcription factors (TFs) of the Snail family are best recognized for being the direct repressors of epithelial morphology promoter *E-cadherin* transcription that drives EMT [9]. Snail family TFs play central role during the embryogenesis processes of both invertebrates and higher order animals where they regulate the cell movements necessary for the formation of the mesoderm [10]. Their involvement in the formation of vertebrate neural crest cells has been appreciated for more than two decades [11]. While Snail induced migratory and invasive behavior in developmental cells is vital for embryonic development, the same becomes problematic when aberrantly activated in later stages especially in pathological states such as cancer [12]. It is well established that enhancement in *Snail* gene expression in primary tumors promotes cellular motility and the consequent acquisition of metastatic properties [13,14]. On the other hand, in non-transformed cells, the enhancement in snail protein expression induces fibrosis like features [15]. Studies have clearly demonstrated that targeted down-regulation of snail can reverse EMT [16]. Human snail is a 264-amino acid nuclear protein with an amino-terminal basic amino acid-rich domain (SNAG domain) and a carboxyl-terminal DNA-binding domain (zinc finger domain) (Fig. 1). The Snail superfamily also includes the Scratch proteins [17]. More than 100 targets of *Snail* have been identified to date from all metazoan groups, with five family members in vertebrates: *Snail1*, *Snail2*, *Snail3* and *Scratch1* and *Scratch2*. The domain structures of all Snail and Scratch TFs is conserved, *i.e.* having a divergent N-terminal half of the protein and a highly conserved C-terminal half as the DNA binding domain (DBD) which contains four to six zinc fingers (ZFs) of the C2H2 type [17] (Fig. 1). The expression of *Snail* family genes is regulated at the transcriptional level by many signaling molecules, including FGF [18], Wnt [19] and TGFβ [20] which collectively form the building blocks of the microenvironment that serves as a niche for EMT [21].

3. Cellular localization of Snail family proteins

As transcription factors that require sequence specific alignment on DNA for gene regulation, snail proteins must translocate to the cell nucleus in order to be functional. Like all the proteins, snail family members cannot passively diffuse through the nuclear membrane and require a carrier for their nuclear import or export. Their translocation requires energy and in most of the cases, it is mediated by importin-β (Impβ/KPNB1) belonging to the karyopherin family proteins [22]. These receptors are 90–130 kDa soluble proteins interacting with the cargo they are going to transport, the transport proteins and the GTPase Ran [23] (Fig. 2). The importins mediate transport between the cytoplasm and the nucleus, interacting with their cargoes that carry specific amino acid sequences called nuclear localization signals (NLSs) [24]. These interactions can be direct or they may be mediated by karyopherin family that recognize and bind to the NLS present in many of the proteins imported by Importin β [25]. The directionality of the nuclear transport is imposed by a gradient formed by RanGTP across the nuclear envelope (higher concentration of RanGTP in the nucleus and a lower concentration in the cytoplasm) [26]. Importins exclusively interact with their cargoes in the cytoplasm and relocate them to the nucleus where they interact with RanGTP. The RanGTP binding in the nucleus causes lowering of the affinity of the importins for their substrates leading to their release. The RanGTP-bound importins relocate in the cytosol where, through the involvement of RanGAP and RanBP1, the GTP bound to Ran is hydrolyzed and Ran is released from the importin [27]. The importin can then start a new cycle of nuclear import. Taken together, these findings show that nuclear transporters (both importin and exportins) play central role in the biology of Snail (or other EMT promoting TFs with NES and NLS). Therefore, modulation of nuclear transport proteins should in principle result in alterations in Snail cellular localization and consequently impact snail mediated EMT signaling.

3.1. Nuclear import mechanisms of snail family proteins

The regulatory mechanisms that promote Snail nuclear import and enhance its stability have been well investigated. Among the earliest studies, Yamasaki and colleagues utilized a number of different fusion proteins containing a green fluorescent protein (GFP) to generate a series of the Snail fragments to analyze their subcellular localization [28]. In their studies, the fusion of the four zinc fingers to GFP led to the targeting of GFP to the nucleus, indicating that the zinc finger domain is sufficient for nuclear localization. More convincing evidence came from experiments where an *in vitro* transport system was used and the nuclear import of Snail was reconstituted by importin in the presence of Ran and NTF2. This approach further demonstrated that Snail binds directly to importin in a zinc finger domain-dependent manner. These results indicated that zinc finger domain of Snail functions as a nuclear localization signal and Snail can be transported into the nucleus in an importin-mediated manner. Interestingly, the above studies also highlighted that all four zinc fingers are necessary for efficient nuclear localization, because removing of any one zinc finger alone or in combination resulted in a decreased nuclear accumulation. It is likely that all four fingers are required for the coordination of the structure of the carboxyl terminal domain to interact efficiently with the nuclear import machinery and actually function as the NLS of Snail. It should be noted that there remains a possibility that deletion of any zinc finger domains or disruption of the ternary structure of zinc fingers may cause a loss of DNA binding ability, resulting in a loss of nuclear retention. Therefore, strategies disrupting the ternary complex or blocking access of the importins to the 4 zinc finger domains can certainly be postulated to influence the nuclear retention and the importin binding activity that may provide opportunity for targeted inhibition of nuclear localization of snail.

3.2. Nuclear exclusion mechanisms of snail

Several mechanisms can explain the effect of the exportin recognizable domains in Snail on its own subcellular localization. These domains may act as an anchor linking Snail to specific cytosolic proteins; they may inhibit its nuclear import, or mediate its nuclear export. It is well known that the most common mechanism of nuclear export of proteins in eukaryotic cells is through Xpo1/CRM1-dependent systems. Most exportable proteins have a hydrophobic Leu-rich sequence and snail is no exception. By searching in this direction, the nuclear exclusion sequence (NES) of mouse Snail protein (aa residues 132–143) was discovered more than a decade ago [29]. It was shown that the specific Snail Leu-rich amino acid sequence is similar to those described for in other CRM1-targeted export proteins. These studies identified the sequence LGQLPKQLARLS, between the aa residues 132–143 of murine Snail, that was shown to perfectly match the consensus sequence previously defined for established NESs [$LX_{(1-3)}LX_{(2-3)}LXL$]. Interestingly, replacement of Leucine with Valine, as commonly observed to occur in human Snail (Leu135 to Val), has been observed in some other NESs, as well as longer

Download English Version:

https://daneshyari.com/en/article/2023691

Download Persian Version:

https://daneshyari.com/article/2023691

Daneshyari.com