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Commentary

Restore the brake on tumor progression

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ABSTRACT

Sonic hedgehog (Shh) signaling plays a key role in regulation of normal development. The negative feedback mechanism mediated by the transcriptional factor, Gli3, acts to finely tune Shh signaling, providing tight control of normal developmental processes. Hyperactivation of Shh signaling often leads to many human malignancies, including basal cell carcinoma and medulloblastoma (MB). However, how tumor cells sustain the aberrant activation of Shh signaling is still not completely understood. We recently revealed that during MB formation, tumor cells express Nestin, a type VI intermediate filament protein, which maintains uncontrolled Shh signaling by abolishing negative feedback by Gli3. Therefore, Nestin expression is a necessary step for MB formation. These findings highlight the novel function of Nestin in regulating Shh signaling, as well as the important role of a disrupted negative feedback mechanism in MB tumorigenesis. Further, restoration of the intrinsic negative feedback by repressing Nestin expression represents a promising approach to treat MB as well as other Shh signaling associated malignancies.

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Contents

1. Introduction

Proper development of multi-cellular organisms relies on the coordination of a diverse array of signal transduction pathways. Most signaling events are required to stay homeostatically active under precise regulation by activating as well as repressing signals. In many cases, negative feedback mechanisms act to dampen signal transduction, preventing aberrant pathway activation.

Negative feedback regulation is particularly important in the Sonic hedgehog (Shh) signaling pathway. Shh signaling plays a fundamental role during normal vertebrate development by regulating tissue patterning, cell proliferation and differentiation, as well as fate determination. Shh ligand is a secreted protein, which acts by activating a protein complex, consisting of transmembrane receptors Patched 1 (Ptch1) and Smoothened (Smo). In the absence of Shh ligand, its receptor Ptch1 inhibits pathway effector Smo, preventing the activation of Shh pathway. When Shh ligand is present, its binding to Ptch1 results in the subsequent activation of Smo, which in turn leads to transcription of Gli zinc-finger transcriptional factors and pathway activation [1]. In mammalian cells,

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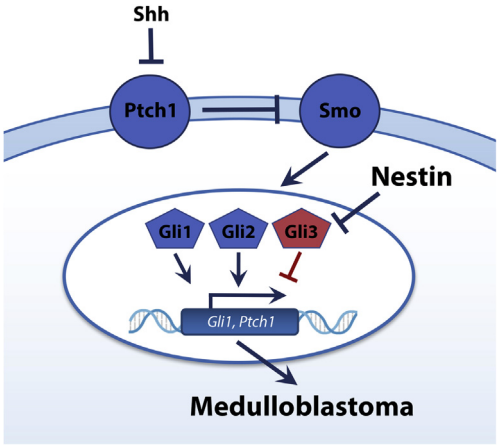

Fig. 1. Nestin augments Shh signal transduction by inhibiting its repressor, Gli3. Shh ligand interacts with its antagonizing receptor Ptch1, which activates the effector protein, Smo, leading to nuclear translocation of the Gli family of transcriptional factors: Gli1, Gli2 and Gli3. While Gli1 and Gli2 act primarily as transcriptional activators, Gli3 blocks subsequent transcription of Shh pathway target genes (*Gli1*, *Ptch1*, etc.), acting as an essential negative regulator of Shh signaling transduction. Recent work from our laboratory has demonstrated that Nestin mediates Shh pathway associated tumorigenesis by abolishing the inhibitory functions of Gli3 on Shh signaling [7].

there are three Gli protein members – Gli1, Gli2 and Gli3, which are normally tethered to the cytoskeleton. Gli proteins are essential effectors of Shh signaling which determine the pathway outcome. The functions of Gli proteins are regulated by proteolytic processing into balanced full-length transcriptional activators or truncated repressor forms. Gli1 and Gli2 are generally considered to be activators of Shh signaling, while Gli3 predominantly imparts a repressive function. Full-length Gli3 is mostly processed into its repressor form, which acts as a major negative regulator of Shh signaling transduction [2,3]. After proteolytic processing, the truncated Gli3 translocates into cell nuclei to repress the activation of Shh pathway (Fig. 1). The negative feedback loop mediated by Gli3 plays an important role in ensuring the activation of Shh pathway at physiologic levels.

In the cerebellum, Shh ligand is released from Purkinje neurons and stimulates the proliferation of granule neuron precursors (GNPs) in the external granule layer (EGL). During the first 2–3 postnatal weeks in mice, GNPs exit the cell cycle, differentiate and migrate inward to form the internal granule layer. In parallel, Shh signaling is gradually down-regulated in GNPs, even in the Shh-enriched environment [4,5]. Aberrant activation of Shh pathway in GNPs caused by loss of Ptch1, drives ectopic proliferation of GNPs. After prolonged proliferation, Shh signaling diminishes in the majority of Ptch1-deficient GNPs. Only a rare population of cells sustains activation of the Shh pathway and ultimately results in the formation of medulloblastoma (MB) [6]. The discrepancy between the normal developmental self-restricted activation of signaling in GNPs and the abnormal proliferation with aberrant pathway activation implies the importance of negative regulation of the Shh pathway. This observation suggests that the disruption of the negative regulation on Shh signaling is critical for MB tumorigenesis.

We have recently found that during MB formation, tumor cells gradually express Nestin, a type VI intermediate filament protein. Nestin expression plays a critical role in MB tumorigenesis by sustaining aberrant Shh signaling in MB cells. To achieve this, Nestin impairs the inhibitory regulation of Gli3, driving MB initiation and progression [7]. Our studies highlight the novel function of Nestin in regulating Shh signaling and the importance of disruption in the negative feedback regulation in the tumorigenesis of MB. In this review, we summarize the expression and functions of Nestin in regulating Shh signal transduction and the negative feedback mechanism in tumorigenesis and the therapeutic implications of the negative feedback.

2. General knowledge of Nestin and its expression in tumors

Nestin, first identified in the neural stem cell (NSC) in 1985, is widely considered a putative marker for stem cells [8,9]. The human Nestin gene encodes a large protein consisting of 1621 amino acids. Structural organization of the Nestin gene is evolutionarily conserved between human, rat and mouse, indicative of its functional significance [10]. Nestin expression is predominantly regulated by enhancer regions in the first and second introns of the Nestin gene [11,12]. A 714 bp conserved 3′ portion of the second intron is sufficient to control Nestin expression in progenitors in the central nervous system. An enhancer region of the Nestin gene is found in the second intron and is divided into two separate domains [13]: the 3′ region required to induce Nestin expression in pan-CNS progenitors and the other controlling expression in the midbrain. The putative binding sites located in the second intron of the Nestin gene include Retinoic Acid Receptor (RAR), Retinoid X Receptor (RXR), and Thyroid Hormone Receptor (TR) [14,15]. In human umbilical vein endothelial cells, Nestin expression was found to be regulated by an element in the first intron [16].

Similar to other intermediate filaments, such as cytokeratin and vimentin, Nestin consists of an α-helical rod domain, flanked by N-terminal 'head' and C-terminal 'tail' domains. The highly conserved rod domain of the Nestin protein contains several α-helical coils that assemble in antiparallel fashion, resulting in filament formation. The alignment of stable dimers and cohesive forces between adjacent dimers determines the properties of high stability and plasticity in the formed Nestin [17,18]. Unlike the majority of other intermediate filaments, Nestin was characterized as having an unusually long C-terminal domain and a relatively short N-terminal domain, which define its self-assembly characteristics [17]. The C-terminal domain gives Nestin a remarkable binding capacity to a wide range of proteins and serves as a platform for cell signal integrations. The short N-terminal domain limits the self-polymerization capability in Nestin, leaving its filament formation to be entirely dependent on interactions with other intermediate filament proteins. It is well established that Nestin often forms complexes with vimentin and α-internexin, allowing formation of stable cytoskeletal intermediate filament networks which maintain the structural integrity of the cells [19–21].

Nestin expression is very dynamic and tightly regulated, both spatially and temporally. Although Nestin is commonly utilized as an NSC marker, it is expressed by a variety of progenitor cells, including skin and hair follicle, muscular, renal, hepatic, endothelial, mesenchymal, hematopoetic and neuronal progenitors [22–31]. In general, the expression of Nestin is primarily correlated with the proliferative stage of such progenitors. As cells exit the cell cycle and undergo terminal differentiation, Nestin expression gradually decreases and is replaced by tissue-specific intermediate filaments, such as glial fibrillary acidic protein in astrocytes, α-internexin and neurofilament in neurons, and desmin in myocytes [32,33]. Nestin expression is normally down-regulated in mature tissues, but its expression can be driven by conditions resembling developmental processes, such as tissue regeneration, revascularization and wound healing. For example, Nestin expression was observed in regenerating muscle tissue following injury or necrosis, as well as in reactive astrocytes during post-injury glial scar formation [34,35]. Nestin expression has also been observed in tissues with pathologic conditions, i.e. in the tooth during carious