A promoter-associated RNA downregulates the oncogenic GLI1 transcription factor in rhabdomyosarcoma cells

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Recent experimental evidence demonstrates a novel regulatory mechanism on the expression of the GLI1 oncogene, a transcriptional effector of Hedgehog signaling. This is mediated by a non-coding RNA, antisense to the GLI1 promoter, GLI1AS, which elicits negative feedback on GLI1 expression. Knockdown of GLI1AS was shown to enhance rhabdomyosarcoma tumor growth in a xenograft model, in-line with the observed increase of the expression levels of GLI1, a known proliferative/oncogenic factor in this cellular context.

Keywords: Non-coding RNA; Gene expression; Bi-directional promoter; Tumor suppressor gene; Chromatin modification

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GLI1 and oncogenic signaling

Glioma-associated oncogene 1 (GLI1) is a terminal effector of Hedgehog signaling that acts to further enhance transcriptional activation, initially elicited by its homologs GLI2 and GLI3. GLI1 is a five-zinc finger protein that is upregulated by Hedgehog signaling and serves as a marker of pathway activity. Among GLI1 targets is GLI1 itself and this generates a positive feedback loop in GLI1 activation [1, 2].

GLI1 is an oncogene and overexpression of GLI1 was shown to result in basal cell carcinoma-like lesions in transgenic mice [3]. On the other hand inactivation of GLI1 has no visible phenotype in mice, however in combination with a GLI2 knockout, more pronounced developmental defects are observed [4]. Moreover, a recent detailed analysis demonstrated the impact of this GLI1 inactivation in embryonic thymocyte differentiatation [5]. Additionally, GLI1 knockdown or small molecule inhibition of GLI1 activity reduces rhabdomyosarcoma cell proliferation and tumor growth in a xenograft model [6]. Similar results, highlighting the oncogenic properties of GLI1, were also observed with neuroblastoma cell proliferation and tumor growth [7].

Promoter-associated RNAs

Recent global analysis has shown that gene promoters generate transcripts not only in the direction of the protein-encoding gene, but also in the opposite orientation (antisense transcription). These antisense transcripts, termed promoter-associated RNAs, are generally considered to be short and unstable, bearing similarities with enhancer-associated transcripts, with little evidence for functionality [8]. However, accumulated recent data indicating that certain promoter-associated RNAs may be longer multi-exon RNAs with defined biological roles are starting to emerge [9-11].

GLI1AS, a functional antisense RNA from the GLI1 promoter

Bioinformatic and experimental analysis has demonstrated...
that a three-exon non-coding RNA from the antisense strand of the GLI1 gene, flanking the GLI1 promoter, which we termed GLI1 antisense (GLI1AS), is expressed in a variety of human cell lines and tumor samples. Moreover, GLI1AS expression was found to correlate well to that of GLI1, with GLI1AS being a target gene of the GLI1 transcription factor. Interestingly, siRNA depletion of GLI1AS or GLI1AS overexpression in rhabdomyosarcoma cells resulted in changes of GLI1 mRNA, an increase and a decrease, respectively. These modulations of the GLI1AS RNA levels, with the concomitant changes in GLI1 expression, had an impact in rhabdomyosarcoma cell proliferation and tumor growth in xenografts on fertilized chicken egg (chorioallantoic membrane CAM assay) [12]. Consequently, the accumulated evidence suggests that GLI1 and GLI1AS are co-regulated, with GLI1AS down regulating the expression of the GLI1 oncogene, acting therefore as a tumor suppressor gene in rhabdomyosarcoma (Figure 1).

Future directions

There are several important questions that should be addressed to further unravel the biological implications of GLI1AS.

1. GLI1AS clearly regulates GLI1 in rhabdomyosarcoma cells and GLI1 is a proliferative/oncogenic factor in these tumor cells. However, it is possible that GLI1AS may affect the expression of other genes as well. Consequently, global analysis to identify possible additional targets of GLI1AS is a priority.

2. GLI1AS appears to act as an epigenetic modulator eliciting repressive chromatin marks at the GLI1/GLI1AS locus, with the polycomb complex apparently involved. This has to be demonstrated by RNA immunoprecipitation analysis. Additionally, cellular factors that may interact with GLI1AS should be identified in unbiased approaches.

3. A structure/function analysis of GLI1AS is highly relevant, as very little is known about functional domains in non-coding RNAs. This may also address whether known polymorphisms in GLI1AS could affect its capacity to act as a tumor suppressor.

4. Examining the role of GLI1AS in additional cellular contexts, especially in other GLI1-dependent cancers, including neuroblastoma, medulloblastoma, breast and pancreatic cancer, is highly relevant.

5. The possibility of exploiting alterations in the GLI1AS/GLI1 expression ratio for cancer therapeutic approaches should be considered.

Conclusion

A three-exon non-coding RNA from the antisense strand of the GLI1 oncogene is not only co-regulated with GLI1 but also represses GLI1 expression, acting therefore as a tumor suppressor gene.

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References


