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AUTHOR'S VIEWS



# NORRIN plays a context-dependent role in glioblastoma stem cells

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**ABSTRACT**

We recently reported a novel role of the atypical Wnt ligand, NORRIN, in mediating the proliferation and stemness of glioblastoma stem cells. Mechanistic and functional analysis revealed context-specific phenotypes in which NORRIN can induce opposite effects on the tumor outcome, depending on the underlying molecular signature of the tumor cells.

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## Article

Glioblastoma (GBM), the most common type of adult brain tumor, is one of the deadliest cancers. Due to its genetic and cellular heterogeneity, genomic instability, fast invasive growth and infiltration, GBM remains largely refractory to current therapeutic regimens and is often referred to as incurable.[1] The standard treatment strategies for GBM include surgical resection followed by adjuvant radio- and chemotherapy, primarily with Temozolomide (TMZ). TMZ is only effective in a fraction of GBM patients, leaving a significant unmet clinical need.[2] Recent research efforts have focused on identifying vulnerabilities of GBM in order to identify novel targets for treatment. Targeting GBM stem cells has surfaced as one of the most exciting and promising therapeutic approaches. Briefly, GBM stem cells (GSCs) are a small population of cells in the tumor that harbor the self-renewal and propagation capacities of the cancer.[3] Thus, eradicating this population was predicted to provide substantial therapeutic advantage for patients. The classical and very well-defined stem cell hierarchy of GBM presents additional vulnerability making this therapeutic approach more interesting.[4] Thus, several trials were undertaken to target GSCs by manipulating the major proteins and pathways that control their biology, such as Wnt, Bone Morphogenetic Protein (BMP), Notch and Hypoxia-Inducible Factor −1 (HIF-1).[5] However; most of these efforts resulted in low success rates, highlighting the urgent need for a better understanding of the molecular processes underlying GSC biology. Additionally, this low rate of success indicates a need for molecular stratification techniques for more precise targeting of GSCs.

Recently, Park and colleagues discovered that the expression level of *Achaete-Scute Family BHLH Transcription Factor 1* (*ASCL1*), a pioneering neural differentiation factor, stratifies GSCs into two cohorts with significantly different growth and differentiation attributes.[6] ASCL1$^{high}$ GSCs harbor the exclusive capacity for terminal neuronal differentiation, and subsequent cell cycle exit and death in response to Notch inhibitors. In contrast, ASCL1$^{low}$ GSCs are resistant to the same class of Notch inhibitors.[6] Besides providing an important insight into the biology of GSCs, this study also highlights the importance of tumor stratification and targeted therapy approaches.

In our recent study, we investigated the role of NORRIN in human GSCs.[7] NORRIN, the protein product of the *Norrie Disease Pseudoglioma* (*NDP*) gene, together with its receptor FRIZZLED-4 (FZD4) have established roles in human congenital eye and inner ear diseases that are characterized by vascular defects.[8] In these contexts, NORRIN functions as an atypical Wnt ligand that signals through FZD4 and co-receptors to activate the canonical beta-catenin/Wnt pathway in endothelial cells[8] (Figure 1). We recently reported that NORRIN signaling in the endothelium is an essential stromal component that regulates tumor initiation in the cerebellum of mouse models of spontaneous Sonic hedgehog meduloblastoma,[9] identifying a role for *NDP/FZD4* in tumorigenesis. In surveying transcriptome data from primary tumors and tumor cell lines we found that *NDP* and *FZD4* are expressed in a wide range of tumor types, and the expression of *NDP*, but not *FZD4*, is markedly enriched in GBM. Moreover, there is a significant correlation between *NDP* expression and patient survival in several neurological cancers including GBM. We used patient-derived GSC lines, a model that has been shown to recapitulate the heterogeneity and cellular biology of primary tumors, to confirm the expression of *NDP/FZD4* pathway components specifically in GSCs. Using gain and loss of function approaches, we uncovered an exciting mechanism in which NORRIN inhibits proliferation and tumor progression of ASCL1$^{low}$ GSCs and exerts the opposite effects on ASCL1$^{high}$ GSCs both in vitro and in vivo mouse xenograft models. We also found that NORRIN only stimulates the stemness of the ASCL1$^{high}$ GSC subtype. In parallel with this phenotypic divergence, RNA-seq analysis revealed common and unique gene programs that are activated or inhibited in both GSC subtypes after *NDP* knockdown. Mechanistic analysis revealed that NORRIN function in ASCL1$^{low}$ GSCs is FZD4- and Wnt-dependent, while in ASCL1$^{high}$ GSCs it is Wnt-independent

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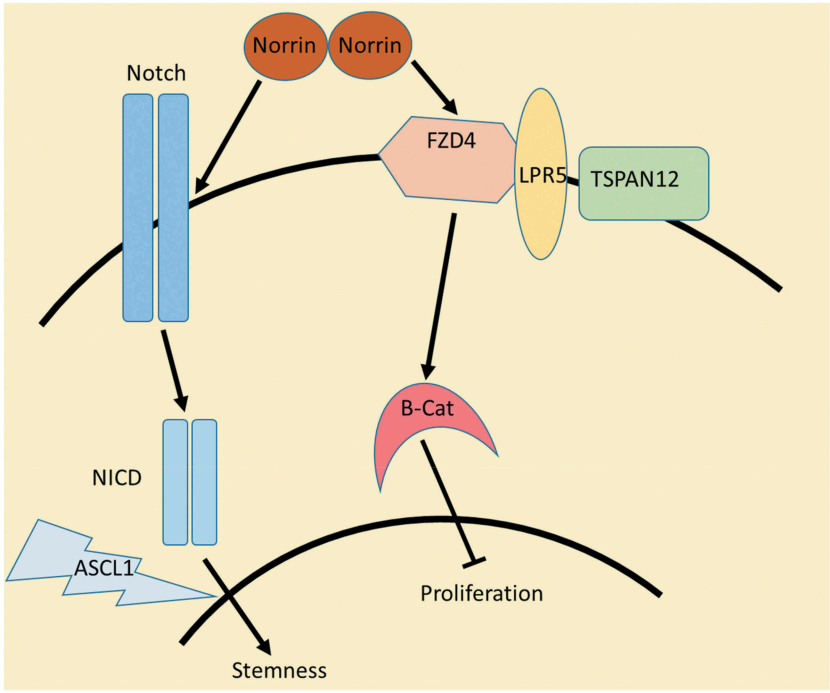


**Figure 1.** Context-dependent role of NORRIN in glioblastoma stem cells. In glioblastoma stem cells (GSCs), NORRIN, the secreted protein encoded by the *Norrie Disease Pseudoglioma* (*NDP*) gene, binds the FRIZZLED-4 (FZD4)/Low-density lipoprotein receptor-related protein 5 (LRP5) receptor complex in the presence of Tetraspanin 12 (TSPAN12) to activate canonical Wnt/Beta-Catenin (B-Cat) signaling, which inhibits proliferation in GSCs expressing low levels of Achaete-Scute Family BHLH Transcription Factor 1 (ASCL1) (ASCL1$^{low}$). NDP also promotes Notch cleavage leading to Notch activation. In ASCL1$^{high}$ GSCs, NORRIN stimulation of Notch signaling through induction of Notch intracellular domain (NICD) promotes self-renewal and tumor progression.

(Figure 1). Because *NDP* knockdown and Notch inhibition induce remarkably similar effects in ASCL1$^{high}$ GSCs, we hypothesized that NORRIN might affect Notch signaling in this context. Strikingly, we found that *NDP* knockdown resulted in a dramatic downregulation of several Notch pathway components including Notch intracellular domain (NICD), Hairy and Enhancer of Split- 1 (HES-1) and Hairy/enhancer-of-split related with YRPW motif-like protein (HEY-L) proteins.[7] Collectively, we show evidence that NORRIN signaling through Wnt and Notch results in context-specific effects on GSC progression (Figure 1).

In the current study we reported an exciting role of NORRIN in controlling GSC progression, presenting a potential therapeutic vulnerability. While the focus of our study was GBM, our analysis of publicly available tumor databases indicated interesting expression patterns of *NDP* in other cancers, such as breast and prostate tumors. Similarly, some of our results indicate a potential role of NORRIN in regulating the proliferation of normal human neural stem cells. Our data presents a clear example of the significance of the cancer cell molecular signature in predicting therapeutic outcomes; for example, treatment with Wnt inhibitors could potentially lead to faster progression of ASCL1$^{low}$ GSCs. This observation is in parallel with reports of a tumor suppressor role of Wnt signaling in specific cellular contexts in GBM.[10] Additionally, we uncover a novel NORRIN function as a Notch regulator, opening new venues for exploring potentially critical roles of this protein in several contexts.

## Disclosure of Potential Conflicts of Interest

The authors declare no potential conflicts of interest.

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