



The development, degeneration and tumorigenesis of the notochord

Journal:	<i>The Innovation Medicine</i>
Manuscript ID	TIME-2026-0084.R3
Manuscript Type:	Review
Keywords:	Notochord, Nucleus pulposus, Chordoma, Targeted therapy, Precision medicine



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

The development, degeneration and tumorigenesis of the notochord

Chencong Lv^{1,2}, Wenxuan Yin^{1,2}, Jianxuan Gao¹, Dianwen Song¹, Tong Meng^{1,*}

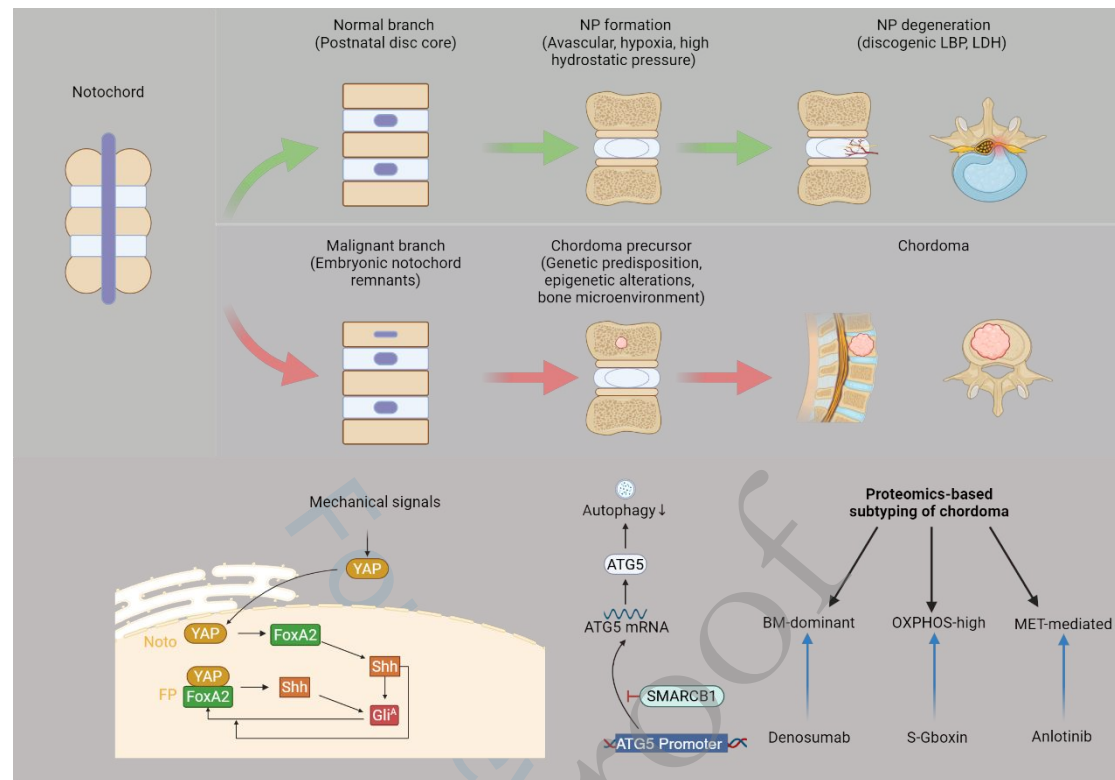
¹ Department of Orthopedics, Shanghai General Hospital, Shanghai Jiao Tong University School of
Medicine, Shanghai, 201620, China

²These authors contributed equally

***Correspondence:** mengtong@medmail.com.cn

Pre-review Only

GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- The notochord can either develop into nucleus pulposus or undergo malignant transformation into chordoma.
- Aging and disc tears drive the loss of notochord cells, leading to inflammation, pain and disc degeneration.
- The bone microenvironment drives chordoma by activating the embryonic notochordal features.
- Targeting this shared origin offers new hope for treating disc degeneration and chordoma.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

ABSTRACT

The notochord is a transient embryonic structure with two distinct postnatal fates. While most notochord cells are compressed into the intervertebral disc to form the nucleus pulposus (NP), incomplete regression leaves remnants in the vertebral body that may serve as chordoma precursors. From this "shared notochord lineage" perspective, this review integrates the regulatory mechanisms governing notochord development, NP formation, and chordoma tumorigenesis. We focus on how the mechanical force-YAP signaling axis and the Shh signaling network coordinately regulate notochord cell fate, and how the aberrant reactivation of these developmental pathways (e.g., brachyury, YAP) drives malignant transformation in chordoma. By bridging embryonic development and cancer biology, this review provides a unified conceptual framework that informs emerging targeted therapies and precision subtyping strategies for chordoma. This integrated perspective highlights how notochord biology determines cell fate and tumorigenesis across these distinct but interrelated contexts.

Pre-review Only

INTRODUCTION

The notochord is a transient embryonic structure that gives rise to two principal postnatal fates. Most notochord cells are compressed into the intervertebral disc to form the nucleus pulposus (NP) of the intervertebral disc (IVD).^{1,2} However, this compression is often incomplete, leaving notochordal remnants within the vertebral bodies. The majority of these remnants remain benign, manifesting as intraosseous notochordal cell tumors, whereas only a small minority undergo malignant transformation. Lineage tracing in mice provides strong evidence that these remnants can serve as the cellular origin of chordoma, a rare and aggressive malignancy.^{3,4} From this “shared notochord lineage” perspective, this review focuses on how key developmental pathways, particularly Brachyury-driven transcription and the mechanical force-Yes-associated protein (YAP) signaling axis, are aberrantly reactivated in chordoma. By integrating recent advances in single-cell transcriptomics, spatial transcriptomics, and organoid models, we elucidate the mechanistic links between notochord development and chordoma tumorigenesis. We further highlight how these insights are informing emerging therapeutic strategies, including brachyury-targeted vaccines, YAP inhibitors, and molecular subtyping-guided precision therapy. Notably, both the NP and chordoma share a notochordal origin,^{3,4} yet previous reviews have largely addressed their development and tumorigenesis as separate topics.⁵⁻⁷ Our framework integrates developmental biology and chordoma oncology to make four core contributions. First, it repositions chordoma as the aberrant end of a continuous developmental trajectory, linking notochord development, NP formation, and chordoma in a single fate map. Second, it proposes a spatiotemporal paradigm where identical signaling pathways yield embryogenesis, homeostasis, or malignancy depending on context, translating shared molecular tools into context-dependent outcomes. Third, it redirects attention from a descriptive common origin to a mechanistic common logic, revealing that the same regulatory code governs both notochord patterning and chordoma maintenance. Fourth, it leverages this mechanistic insight to anchor molecular subtyping in developmental pathway dependencies, moving subtype-guided therapy from descriptive genomics to developmental biology. To our knowledge, this is the first framework to systematically unify a continuous fate trajectory, spatiotemporal dynamics, regulatory circuitry, and molecular subtyping for chordoma, effectively shifting the field from descriptive genomics to mechanistic developmental oncology.

This narrative review is based on literature searches in PubMed and Web of Science (up to May 2026) using keywords such as “notochord”, “nucleus pulposus”, “chordoma”, “Brachyury”, “YAP”, “Shh”, “targeted therapy”, and “immunotherapy”. For established developmental mechanisms (e.g., Shh gradient in neural tube patterning), we cited seminal earlier studies. We prioritized peer-reviewed original research and reviews directly relevant to the “shared notochord lineage” framework, with emphasis on recent findings (last five years) for chordoma biology and therapy, while citing classic studies for established developmental mechanisms.

THE EMBRYONIC DEVELOPMENT AND FATE DETERMINATION OF THE NOTOCHORD

Morphogenesis and structure establishment

The notochord is a transient rod-shaped structure that forms during gastrulation (Figure 1).⁵ It comprises two cell types: inner vacuolated cells, which generate hydrostatic pressure, and outer sheath cells, which secrete an extracellular matrix (ECM)-based basement membrane.⁸ This design makes the notochord a hydrostatic skeleton that drives elongation along the anterior-posterior axis and provides mechanical support during development.⁶ Notch signaling controls the balance between vacuolated and sheath cells,^{8,9} and mechanical coupling between the notochord and paraxial mesoderm coordinates their development.¹⁰

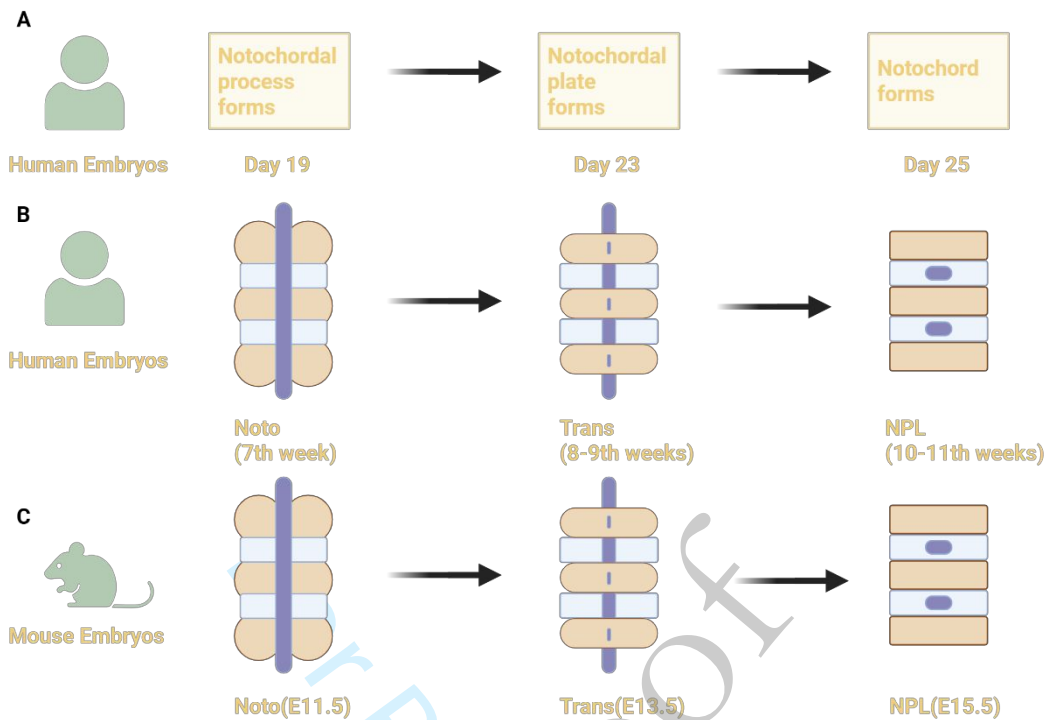


Figure 1. Notochord at Different Stages

(A) Around day 19 of embryonic development, mesenchymal cells from the primitive node migrate cranially, generating notochordal process. By day 23, the notochordal plate further forms, and by day 25, the notochord is fully developed. (B) In human embryos, the notochord stage (Noto) is present at 7 weeks of gestation, with the notochord indicated in purple and the forming or developing vertebrae in brown. The transition stage (Trans) spans weeks 8-9, and the nucleus pulposus-like stage (NPL) is reached by weeks 10-11. (C) In mouse embryos, at embryonic day 11.5 (E11.5), the notochord stage (Noto) is evident, with the notochord represented in purple and the forming or developing vertebrae in brown. The transition stage (Trans) occurs at E13.5, and the nucleus pulposus-like stage (NPL) is observed at E15.5. (Abbreviations: Noto, the notochord stage; Trans, the transition stage; NPL, the nucleus pulposus-like stage)

Notochord cell fate regulatory network

Upstream inputs: mechanical force and YAP. Atomic force microscopy (AFM) revealed a stiffness gradient in the developing neural tube, peaking in the notochord and the ventral region.¹¹ This mechanical cue activates YAP, a classical mechanotransducer. Activated YAP translocates to the nucleus, binds TEAD factors, and initiates downstream gene expression. Importantly, YAP plays stage- and context-dependent roles in notochord development. During early notochord fate specification, timely inactivation of YAP, together with FGF-mediated MAPK signaling, promotes WNT activation and induces *TBXT* (Brachyury) expression.

Persistent YAP activation at this stage suppresses *TBXT*, indicating that a precise temporal window of YAP inactivation is critical for notochord lineage commitment.¹² During later notochord morphogenesis and neural tube patterning, YAP activation in the notochord and floor plate supports mechanical signaling, upregulates FoxA2, and cooperates with FoxA2 to activate Shh expression (Figure 2).¹¹ This tightly regulated, stage-dependent YAP circuitry is frequently hijacked in chordoma, where sustained YAP activation forms a regulatory axis with Brachyury, driving tumorigenesis, therapy resistance, and tumor stemness.¹³

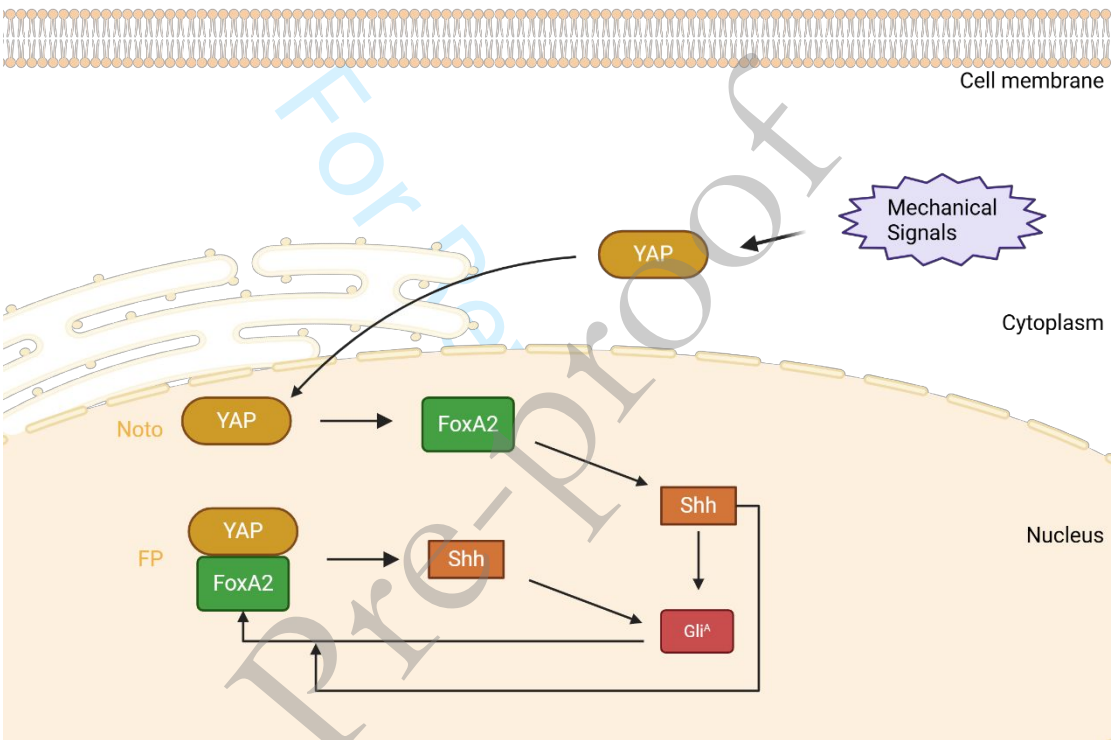


Figure 2. Regulation of Shh Expression by YAP

Upon receiving mechanical signals, YAP is activated in the cytoplasm, translocates to the nucleus, and promotes the expression of FoxA2 in the notochord, which subsequently enhances the expression of Shh. Additionally, YAP can synergize with FoxA2 to augment Shh expression in the floor plate. FoxA2 is activated by Shh produced in the notochord or later through activated Gli transcription factors, creating a positive feedback loop. These mechanisms are supported by studies in mice. (Abbreviations: YAP, Yes-associated protein 1; FoxA2, Forkhead box protein A2; Noto, Notochord; FP, floor plate; Shh, Sonic hedgehog; Gli^A, activated Gli transcription factors)

Core regulators: Shh, FoxA2 and Brachyury. The notochord acts as a key signaling center by secreting Sonic hedgehog (Shh). Shh establishes a concentration gradient along the dorsoventral axis of the neural tube. Importantly, both the concentration and duration of Shh

exposure determine ventral neuron identities.¹⁴⁻¹⁶ The floor plate, the most ventral structure, requires the highest Shh levels and prolonged exposure. Progressively lower Shh concentrations and shorter exposure times induce ventral interneurons and motor neurons at more lateral positions.¹⁶ This Shh-mediated patterning is recapitulated in human trunk-like structures lacking the notochord, in which the neural tube exhibits pronounced dorsalization.¹⁷ Beyond its role in neural patterning, Shh signaling also operates within the notochord itself, where it contributes to notochord cell survival and sheath formation.¹⁸

FoxA1 and FoxA2 are forkhead box transcription factors highly expressed in the notochord.¹⁹ Studies show that YAP signaling upregulates FoxA2, facilitates notochord formation, and collaborates with FoxA2 to activate Shh signaling, which in turn induces floor plate development (Figure 2).¹¹ Double-mutant mice with notochord-specific conditional knockout of *Foxa2* and global knockout of *Foxa1* exhibit multiple developmental defects: abnormal notochord development, loss of Shh expression, and improper formation of the NP.¹⁹ Meanwhile, FoxA2 itself is a downstream target of Shh signaling, potentially forming a positive feedback loop in the floor plate that stabilizes the signal output.¹¹

Brachyury, encoded by the T-box transcription factor T (*TBXT*) gene, is another key transcription factor of the notochord. Its homozygous mutations can result in notochord loss and disrupt primitive streak formation and axial elongation.²⁰ A study identified a 37 kb region upstream of *TBXT*, containing a crucial T-bound notochord enhancer (TNE) essential for notochord function and tailbud outgrowth. Deletion of this TNE leads to abnormal tail bud formation, resulting in tail truncation and impaired axial development.^{21,22}

The divergence of notochord fate differentiation

During spine formation, the notochord gradually becomes confined to the IVD and differentiates into the NP. Lineage tracing studies confirm that NP cells originate from notochord cells.^{3,23} However, this compression is sometimes incomplete, leaving rare isolated embryonic notochord cells within the vertebral bodies. These remnants are considered the cellular origin of chordoma, although most remain benign.²⁴ Thus, the distribution of notochord cells during late embryonic development determines their distinct fates: those that enter the IVD differentiate into the NP, whereas those that remain within the vertebral bodies may serve as the origin of chordoma.

TRANSITION FROM NOTOCHORD TO NP: FROM HYDROSTATIC SKELETON TO DISC CORE

During late embryonic development, the notochord undergoes marked morphological remodeling as the vertebral bodies form. The notochord within the vertebral bodies degenerates, while that within the IVDs persists as the NP. This transition represents a critical branching point in notochord fate, shifting its role from an embryonic axial hydrostatic skeleton to a postnatal load-bearing capacity.

Morphology and dynamics during the transition

Two main models explain how notochord cells are confined to IVD regions. The pressure model proposes that compressive forces drive notochord cells into the intervertebral spaces. This model is further supported by mutant mice lacking a notochord sheath yet possessing normal vertebrae, in which notochord cells are dispersed throughout the vertebral bodies.^{18,25} The repulsion-attraction model proposes that notochord cell distribution is guided by molecular cues: attraction within IVD regions and repulsion within the vertebral bodies.²⁶ These models may operate concurrently; however, direct measurement of mechanical pressure on the notochord is needed to distinguish their relative contributions.

Molecular reprogramming and cellular heterogeneity

Whole-transcriptome analysis of the notochord-to-NP transition revealed a shift from active Shh/Wnt signaling to upregulated TGF- β /IGF pathways and ECM components.²⁷ Single-cell RNA-seq identified at least two notochord-derived subpopulations spanning a continuum from notochord-like to chondrocyte-like cells, with activation of TGF- β and PI3K-AKT signaling.²⁸ Spatial transcriptomics further resolved outer and inner NP subpopulations, with progenitor markers enriched at the periphery.^{29,30} Together, these techniques have reshaped our understanding of NP heterogeneity and delineated a fine molecular map of the notochord-to-NP transition, offering new perspectives on the cellular origin and functional maintenance of the NP.

Consistent with its role in NP maintenance function, the NP resides in an avascular microenvironment characterized by low oxygen tension and high hydrostatic pressure.³¹ Transcriptome analysis showed that HIF-1 α is continuously expressed in notochord-derived NP cells during the notochord-to-NP transition,²⁷ and it is essential for NP cell survival under

hypoxia by upregulating glycolytic key enzyme PGK-1.³¹ Additionally, FOXO transcription factors directly regulate autophagy, hypoxia adaptation, and resistance to oxidative stress in human NP cells, thereby mediating NP maturation and maintenance.³² Thus, the transition from notochord to NP involves reprogramming from an embryonic signaling state to an ECM synthesis state adapted to the hypoxic microenvironment.

Outstanding controversies

Despite these models, the precise mechanisms governing notochord cell migration into the NP remain not fully understood. The pressure model lacks direct *in vivo* measurements of the compressive forces proposed to drive this migration. The attraction model has identified attractive cues such as the SDF-1 α /CXCR4 axis in bone marrow-derived mesenchymal stem cells (BMSCs),³³ but whether analogous mechanisms confine notochord cells to the IVD remains unknown. Additionally, the identity of putative repulsive signals that prevent their entry into vertebral bodies has yet to be molecularly characterized.

Furthermore, the relative contributions of mechanical versus molecular guidance remain poorly defined. While YAP-mediated mechanotransduction is critical for notochord formation,¹¹ it remains unclear whether tissue stiffness gradients directly guide cell migration or primarily modulate gene expression, thereby controlling chemotactic responses. Addressing these questions will require combining quantitative *in vivo* force measurements with targeted genetic perturbations of mechanotransduction components in migrating notochord cells.

In addition, the extent to which current animal models recapitulate human notochord development remains a limitation. Most mechanistic insights have been derived from mouse, chick, and zebrafish embryos, yet important species differences exist. Improved models, including recently developed human trunk organoids that contain a functional notochord,¹² are beginning to bridge this gap and provide new platforms to address these outstanding questions.

Degeneration of notochord-derived cells in the NP and stem cell replacement therapy

Postnatally, notochord-derived NP cells gradually decline in number with age and are replaced by chondrocyte-like cells.³⁴ Beyond the rate of this physiological transition, intervertebral disc degeneration (IVDD) involves the depletion of notochord-derived TAGLN⁺ progenitor cells and the loss of their trophic "housekeeping" functions, thereby impairing NP regeneration and homeostatic maintenance.^{2,29,35,36} Key features of this degenerative process include

proteoglycan loss, reduced hydration, inflammation, neovascularization, and sensory nerve ingrowth into the NP, contributing to discogenic low back pain (LBP).³⁷ Additionally, progressive structural failure of the annulus fibrosus (AF) may permit lumbar disc herniation (LDH), mechanically compressing nerve roots (Figure 3).^{38,39}

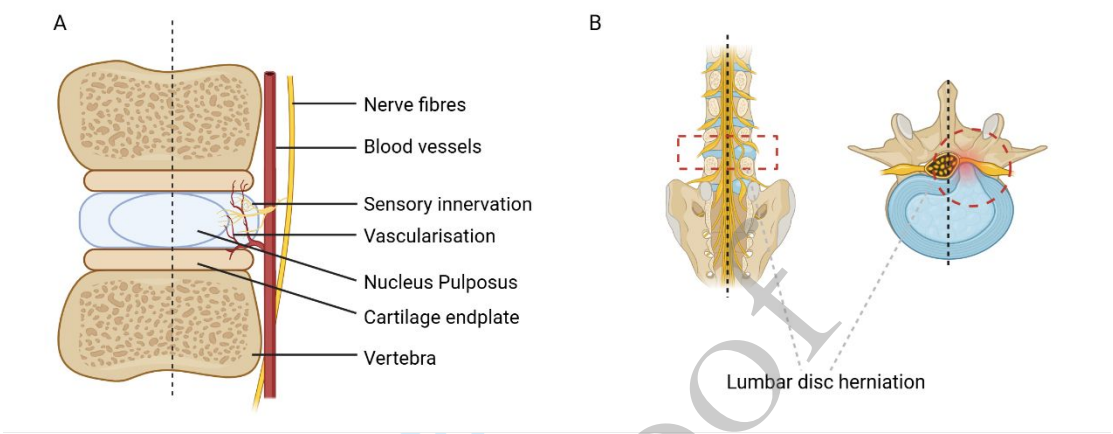


Figure 3. Morphological Changes in Discogenic Low Back Pain and Lumbar Disc Herniation

(A) Blood vessels and sensory nerves grow into the normally avascular and aneural NP of the IVD, leading to discogenic back pain. (B) The NP shifts through the AF, leading to narrowing of the central canal or neural foramina, compressing mechanically the spinal cord or associated nerve roots.

For the treatment of IVDD, replenishing notochord-like cells has been explored in both animal models and early-phase human trials. Some small, uncontrolled clinical studies have reported that autologous BMSCs injected into the degenerated IVD may relieve pain and increase disc water content in some patients with chronic LBP.^{40,41} Additionally, induced pluripotent stem cells (iPSCs) can be differentiated into notochord-like cells and have shown therapeutic benefits in a pig IVDD model.² However, these preclinical and early clinical studies have several key limitations. First, most evidence derives from small, uncontrolled pilot studies with short follow-up durations, limiting the generalizability of the reported benefits in pain relief and disc hydration.^{40,41} Second, the clinical translation of BMSCs faces major obstacles, including poor cell survival and retention within the harsh NP microenvironment (low oxygen tension, high hydrostatic pressure, and nutrient deprivation), as well as the risk of impaired functional differentiation imposed by the degenerated disc niche.⁴² Third, while iPSC-derived notochord-like cells offer a compelling alternative, challenges persist regarding differentiation fidelity,

tumorigenicity, manufacturing standardization, and long-term safety.⁴³ Fourth, the absence of standardized protocols for cell sourcing, dosing, delivery routes, and patient selection criteria hampers cross-study comparisons.⁴⁴ Collectively, these limitations underscore the urgent need for larger, randomized controlled trials with extended follow-up, alongside rigorous mechanistic studies, before cell-based therapies can be considered for clinical translation in IVDD.

NOTOCHORD TUMORIGENESIS: THE MALIGNANT BRANCH OF NOTOCHORD FATE

Notochordal remnants: from normal development to malignant transformation

Incomplete compression of the notochord leaves notochordal remnants within the vertebral bodies. These remnants are detected in approximately 20% of adult spines at autopsy.⁴ The vast majority remain benign, whereas a small minority, under the influence of genetic alterations and the bone microenvironment, undergo malignant transformation into chordoma.^{7,45,46} Thus, the notochord not only gives rise to the NP (the normal branch) but also serves as the precursor of chordoma (the malignant branch).

This raises a key question: why do most notochord remnants remain quiescent or benign, whereas a few undergo malignant transformation? Existing evidence suggests that chordoma development may involve genetic predisposition, epigenetic alterations, and microenvironmental factors.^{7,45,47} In familial chordoma, *TBXT* gene amplification is observed, whereas sporadic cases often harbor deletions of tumor suppressor genes such as *CDKN2A* and *PTEN*, as well as deletion of chromatin subfamily B member 1 (*SMARCB1*).⁷ These alterations may collectively confer proliferative advantages and a differentiation blockade to notochordal remnant cells. Furthermore, integrative multi-omics analysis has revealed that chordoma cells exhibit skeletal development-related features at the levels of the transcriptome, chromatin accessibility, and three-dimensional genome architecture.⁴⁵ Clinical imaging shows that chordoma occurs predominantly in bone tissue rather than in the IVD, suggesting that the bone microenvironment may play a key driving role in chordoma development.⁴⁵

Molecular mechanisms of malignant transformation

Chordoma shares conserved histological and anatomical features with the embryonic notochord, strongly supporting its origin from persistent notochord cells. Anatomically, chordoma exhibits

a striking predilection for the clivus and sacrococcygeal region, corresponding to the typical distribution of embryonic notochordal remnants.⁵ Moreover, chordoma retains core notochordal functions that are lost in the postnatal NP.⁴⁵ This functional conservation, together with shared molecular markers such as Brachyury, underscores the enduring notochord-like identity of chordoma. At the molecular level, chordoma tumorigenesis involves a complex interplay among aberrant reactivation of developmental pathways, epigenetic and genomic alterations, and immune evasion mechanisms that collectively drive malignancy.

The Brachyury-YAP regulatory axis. Brachyury, a master regulator of notochord development, is a highly sensitive and specific diagnostic marker for chordoma. It is expressed in conventional and poorly differentiated subtypes but lost in dedifferentiated components. This consistent expression across most subtypes establishes Brachyury as a key therapeutic target.^{7,48} Its gene amplification observed in familial chordoma points to *TBXT* amplification as a key genetic driver.⁷ Furthermore, chordoma cells exhibit oncogene addiction to Brachyury; experimental knockdown of *T* suppresses tumor cell growth, induces apoptosis, and reduces tumor formation in chordoma xenograft models, underscoring its functional dependency.^{13,48} Mechanistic studies showed that Brachyury promoted tumor invasion by controlling YAP synthesis and stability,¹³ and formed a regulatory axis with fibroblast growth factor receptor (FGFR) signaling to drive tumor cell growth and survival.⁴⁸ However, targeting Brachyury directly presents a major challenge. As a ligandless transcription factor, Brachyury has historically been considered "undruggable" due to its lack of traditional small-molecule binding pockets.⁴⁹ This has prompted alternative strategies, including targeting Brachyury's upstream regulators,⁵⁰ using Brachyury-targeting cancer vaccines,⁵¹ and designing ankyrin repeat proteins that block *TBXT*-DNA binding.⁵² However, recent structural insights and fragment-based screening have challenged this notion by identifying small-molecule ligands that bind to Brachyury's DNA-binding domain, demonstrating that it is ligandable.⁴⁹ While these early-stage compounds are not yet clinical-grade drugs, they provide a crucial proof of concept and open a new direct pharmacological avenue alongside the established alternative strategies. Notably, YAP itself, a mechanotransducer involved in notochord development (see Section 2.2.1), is aberrantly activated in chordoma, and together with brachyury, sustains tumor stemness. However, systemic inhibition of YAP raises substantial safety concerns, given the

essential roles of the Hippo pathway in normal tissue homeostasis. Germline deletion of *Yap*, *Taz*, or *Teads* in mice results in embryonic lethality or severe developmental abnormalities.⁵³ Moreover, YAP/TAZ activity is not uniformly tumor-promoting; rather, it is context-dependent and can even exert tumor-suppressive functions in certain cell types.⁵⁴ These findings underscore the need for strategies that achieve tumor-selective YAP inhibition while minimizing on-target toxicity in normal tissues.

Additional genetic and signaling drivers. Beyond Brachyury-YAP signaling, chordoma tumorigenesis is driven by a convergence of additional molecular alterations, including chromatin remodeling abnormalities, SMARCB1 loss, epigenetic reprogramming, and genomic instability, which collectively establish a permissive landscape for the aberrant reactivation of notochordal developmental programs.

Chromatin remodeling and SMARCB1 loss. The SWI/SNF chromatin remodeling complex, a master regulator of gene accessibility, is frequently disrupted in chordoma through mutations in genes such as *ARID1A* and *PBRM1*.⁵⁵ SMARCB1, a core subunit of the SWI/SNF complex, is specifically lost in poorly differentiated chordomas.⁵⁶ Mechanistically, SMARCB1 represses *ATG5* transcription, thereby regulating autophagy and exerting a protective effect against tumor progression.⁴⁷ This chromatin remodeling defect is not an isolated event; it alters chromatin accessibility and may thereby permit the aberrant reactivation of notochordal developmental programs.⁵⁵ Indeed, Brachyury has been shown to recruit the acetyltransferase p300 to its target gene loci, increasing H3K27ac marks and maintaining an accessible chromatin state during development.⁵⁷ Whether SWI/SNF dysfunction, such as SMARCB1 loss, disrupts this cooperative regulation in chordoma, thereby lowering the threshold for developmental gene activation and contributing to the aggressive phenotype of poorly differentiated chordoma, remains an important open question.

Epigenetic reprogramming. Beyond SWI/SNF-mediated chromatin remodeling, chordoma exhibits broader epigenetic dysregulation. DNA methylation profiling has defined two distinct epigenetic subtypes of skull base chordomas: subtype C, characterized by global hypomethylation with concurrent CpG island hypermethylation, upregulation of cell cycle genes, and heightened chromosomal instability; and subtype I, distinguished by global hypermethylation and an immune-enriched microenvironment.⁵⁸ Collectively, these epigenetic

alterations, together with chromatin remodeling defects, establish a permissive epigenetic landscape that facilitates the reactivation of notochordal developmental genes while concurrently silencing differentiation programs.⁵⁸

Genomic instability. Chordoma is typified by complex chromosomal copy number alterations, alongside a low mutation burden.⁵⁹ Non-random copy number losses are the genomic hallmark, commonly involving chromosomes 1p, 3, 9p, 10, 13, 14, and 18.^{60,61} Gain of chromosome 1q is frequently observed and correlates with chromosomal instability and upregulated mitochondrial functions.⁶² Proteogenomic analyses further link chromosomal instability to immune-cold tumor microenvironments, particularly in subtypes harboring 9p/10q loss. *TP53* mutations, although rare at initial presentation, are acquired during tumor progression and are associated with more aggressive phenotypes.⁶³ Collectively, these genomic alterations may synergize with Brachyury-YAP-driven transcriptional programs to promote malignant transformation.

Additional signaling drivers. *SOX9*, a transcription factor essential for chondrogenesis and notochord development, is overexpressed in chordoma, where it correlates with poor prognosis. Functional studies have demonstrated that *SOX9* knockdown inhibits chordoma cell growth and induces apoptosis and cell cycle arrest.⁶⁴ Beyond individual candidate genes, multi-omics approaches have further expanded the molecular landscape of chordoma. A comparative multi-omics study of chordoma and NP unveiled the role of the bone microenvironment in chordoma progression. Carbonic anhydrase II (CA2) was identified as a novel therapeutic target for chordoma. Indeed, CA2 ablation via genetic knockout or dorzolamide HCl significantly suppressed cell growth and migration of chordoma cells.⁴⁵ At the single-cell level, transcriptomic profiling of skull base chordoma has revealed that malignant cells highly express features associated with partial epithelial-mesenchymal transition (p-EMT). YL-13027, a p-EMT inhibitor that blocks the TGF- β signaling pathway, demonstrated therapeutic efficacy in UM-Chor1 cell line and xenograft models. In a phase I clinical trial that enrolled three skull base chordoma patients, this treatment achieved stable disease with no serious adverse events.⁶⁵

Immune-related mechanisms and emerging targets. Immune evasion is a hallmark of chordoma progression, driven by at least four distinct mechanisms: 1) immune checkpoint

upregulation that dampens T-cell activity;⁶⁶ 2) recruitment of immunosuppressive cells that fosters a pro-tumoral microenvironment;⁶⁷ 3) T-cell exclusion mediated by fibrovascular septa that restrict immune cell infiltration into tumor lobules;⁶⁷ and 4) functional silencing of the cGAS-STING pathway linked to 9p/10q co-deletion, which impairs antigen presentation and immune cell recruitment, thereby contributing to an immune-cold phenotype.^{62,67}

The chordoma microenvironment contains abundant immune cell infiltration, with regulatory T cells (Tregs) and M2-polarized tumor-associated macrophages (TAMs) comprising a substantial proportion.⁶⁸ The CCL5-CCR5 axis plays a key role in regulating macrophage recruitment and M2 polarization, thereby promoting chordoma progression, and has been validated as a potential therapeutic target.⁶⁹ Furthermore, the PD-1/PD-L1 axis has been highlighted as a major immune checkpoint in chordoma, given the high frequency of PD-L1 expression in patient samples.⁶⁶ PD-1/PD-L1 engagement directly suppresses T-cell effector functions, representing a canonical immune evasion pathway. Single-cell analyses have further identified additional immune-related dependencies, including the PD-1/PD-L1 axis.⁶⁵ However, the chordoma immune landscape is markedly heterogeneous, spanning immune-hot to immune-cold phenotypes. This immune heterogeneity is partly driven by genomic alterations such as chromosome 9p/10q loss, which correlates with immune-cold microenvironments. Preclinical studies in chordoma cell lines have also suggested the potential for NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) against chordoma cells using the anti-PD-L1 antibody avelumab.⁷⁰ Nevertheless, the clinical relevance of these targets remains to be established, as most evidence is derived from small case series or preclinical models.^{68,71} Given this heterogeneity, patient selection, potentially guided by genomic features and immune infiltration patterns, and combination strategies will likely be critical for successful immunotherapy.

Research models: from mechanistic exploration to drug screening

Significant advances in chordoma modeling have provided a strong impetus for mechanistic exploration and drug screening.⁷² Multiple chordoma models derived from different cell lines have been successfully established, covering primary, recurrent, and metastatic chordoma.⁷² The 3D cell culture system, which mimics *in vivo* growth conditions *in vitro*, is currently regarded as a significant advancement in cancer cell modeling.^{72,73}

Xenograft models, generated by implanting tumor cell lines or patient-derived tumor cells into

immunodeficient animals, are regarded as the gold standard for assessing the *in vivo* efficacy of potential cancer therapies. A PDX model derived from patient-derived chordoma cells successfully recapitulated key features of the parental tumor, including activation of the mTOR pathway. This model revealed aberrant mTOR pathway activity in chordoma and highlighted the mTOR inhibitor MLN0128 as a potential drug for suppressing chordoma growth and recurrence (Figure 4).⁷⁴ However, PDX models lack a fully intact human immune system and cannot faithfully replicate the human tumor microenvironment, thereby limiting their utility in the evaluation of immunotherapies.

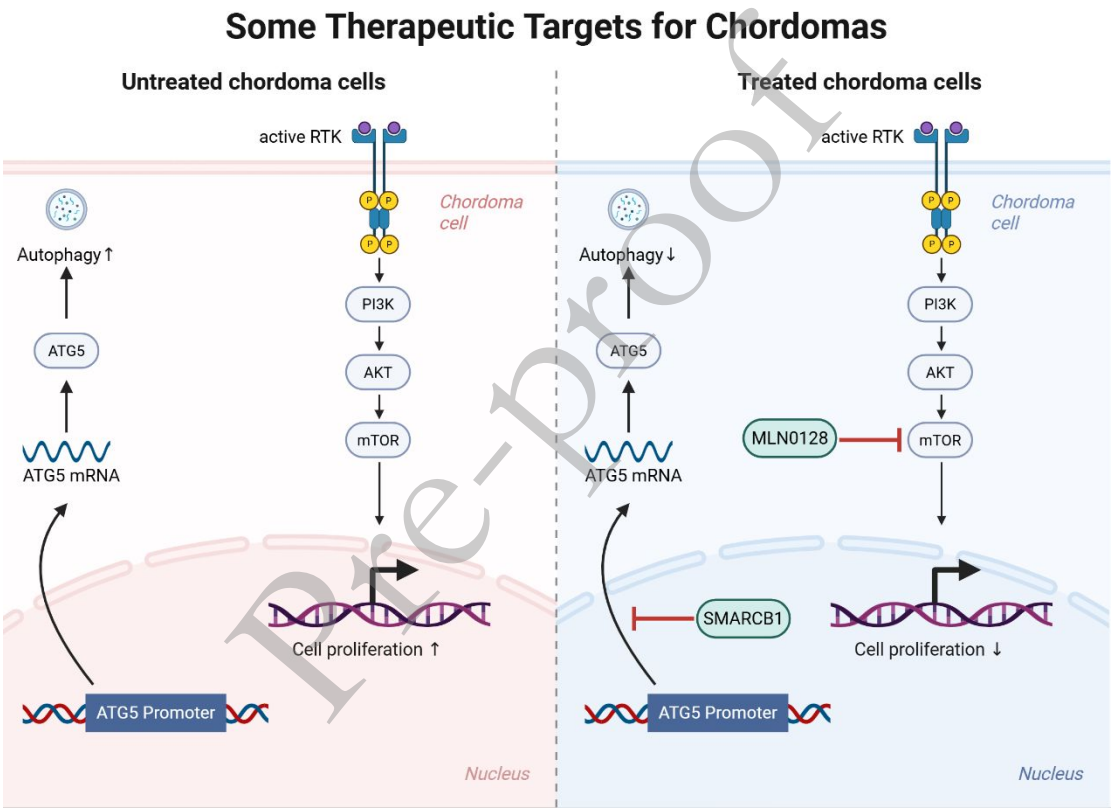


Figure 4. Some Therapeutic Targets for Chordomas

The ATG5 promoter initiates ATG5 transcription, which in turn promotes autophagy to maintain intracellular homeostasis in chordoma cells. SMARCB1 directly binds to the promoter region of ATG5 and epigenetically inhibits ATG5 transcription, thereby suppressing autophagy. Upon activation, RTK triggers a series of signal transduction processes to activate PI3K, which then activates AKT. The activation of AKT further activates mTOR, leading to the growth and recurrence of chordomas. The mTOR inhibitor MLN0128 can inhibit the activity of the mTOR pathway, thereby suppressing the growth and recurrence of chordomas. (ATG5: autophagy-related gene 5; SMARCB1, subfamily B member 1; RTK, Receptor Tyrosine Kinase; AKT, Protein Kinase B; mTOR, Mammalian Target of Rapamycin)

In addition, a transgenic zebrafish model of chordoma was successfully generated through notochord-specific expression of *HRASV12*. This model closely recapitulates human chordoma in histology, ultrastructure, and immunohistochemical features, providing a convenient platform for high-throughput drug screening.⁷⁵

Patient-derived chordoma organoid models have also emerged as complementary platforms that better preserve intratumoral heterogeneity and 3D architecture, although their long-term stability and reproducibility require further optimization. Nevertheless, current models face several limitations, including the lack of immune and vascular components,^{76,77} batch-to-batch variability in ECM scaffolds,⁷⁸ and the static nature of conventional culture systems, which cannot replicate dynamic biomechanical cues.⁷⁹

Treatment strategies: from local control to precision subtyping

As understanding of chordoma molecular mechanisms deepens, treatment strategies are evolving from traditional local control toward targeted therapy, immunotherapy, and precision subtyping. This evolution reflects the translational application of the “notochord lineage perspective” in cancer treatment.

Local therapy: surgery and radiotherapy. Due to the limited sensitivity of chordoma to conventional radiotherapy and chemotherapy, surgery remains the therapeutic cornerstone for chordoma.⁸⁰ However, chordomas are often located in deep sites such as the skull base and sacrococcygeal region, making complete resection difficult and leading to high postoperative recurrence rates.

Advancements in radiation therapy have introduced promising alternatives. Proton therapy, for instance, enables precise delivery of higher radiation doses to the tumor while minimizing damage to surrounding healthy tissues, making it an emerging therapeutic option for chordoma.⁸¹ Carbon ion beams offer unique physical and biological advantages, including a high relative biological effectiveness and a low oxygen enhancement ratio, yielding high therapeutic efficacy with low toxicity. Multiple studies have demonstrated favorable clinical outcomes in patients with chordoma, and carbon ion radiotherapy is now considered a preferred option for advanced, unresectable chordoma in specialized centers.⁸² Nevertheless, both proton and carbon ion therapies remain unavailable at most institutions due to high infrastructure costs, and long-term toxicity data are still being collected.^{83,84} Furthermore, patient selection criteria,

and optimal dose and fractionation schedules require further standardization.⁸⁵

Targeted therapy: targeting chordoma-specific molecules. Receptor tyrosine kinases (RTKs) are frequently overexpressed and activated in chordoma, such as PDGFR, EGFR, FGFR, VEGFR, and c-Met, with downstream PI3K/AKT and RAS/MAPK pathways also upregulated.⁸⁶⁻⁸⁹ These findings establish RTKs as key therapeutic vulnerabilities and provide the molecular rationale for evaluating tyrosine kinase inhibitors (TKIs), the most extensively investigated class of targeted agents in chordoma.

Apatinib, a selective VEGFR2 inhibitor, was evaluated in a single-arm phase II study involving 30 patients with advanced chordoma. In the trial, the median progression-free survival (PFS) was 18 months. The objective response rate (ORR) was 25.9% by Choi criteria, compared with 3.7% by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.⁹⁰ Building on this experience with apatinib, the same group subsequently reported a phase II trial of camrelizumab (anti-PD-1) combined with apatinib in refractory chordoma (N = 33), achieving an ORR of 24.2% by RECIST and a median PFS of 28 months.⁹¹

Lapatinib, a dual EGFR/HER2 TKI, was assessed in a phase II trial of 18 patients with EGFR-positive chordoma. The study met its primary endpoint, achieving a Choi partial response (PR) rate of 33.3%, although no PR was observed per RECIST. The median PFS was 6 months (Choi) and 8 months (RECIST), with a clinical benefit rate of 22%, indicating modest antitumor activity.⁹²

Imatinib, a multi-kinase inhibitor targeting PDGFR and KIT, has been used off-label for chordoma. A systematic review of case series reported that when restricted to the RECIST-reporting subset (4 studies; 181 evaluable patients), the pooled PR rate by RECIST was only 2.2% (4/181), whereas stable disease was observed in 73.5% (133/181). However, the level of evidence remains low (derived largely from phase II trials and retrospective series), and imatinib is not specifically approved for this indication.⁹³

Beyond TKIs, other targeted strategies have been explored. Palbociclib, a CDK4/6 inhibitor, demonstrated modest activity in a phase II single-arm trial of 28 patients with advanced chordoma harboring CDKN2A or p16 loss. The RECIST disease control rate was 39%, with no objective responses observed. Median PFS was 5.6 months, and the safety profile was manageable.⁹⁴ The mTOR inhibitor nab-rapamycin (ABI-009) was evaluated in combination

with nivolumab (anti-PD-1) in a completed phase I/II trial for advanced sarcoma, including chordoma (NCT03190174). The combination exhibited a manageable safety profile, with 100 mg/m² established as the recommended phase II dose (RP2D). In the planned expansion cohort (n = 25 across sarcoma subtypes), promising antitumor activity was observed, with a 24week PFS rate of 88%, warranting further investigation in chordoma. Direct therapeutic targeting of the Brachyury (*TBXT*) protein, exemplified by agents such as T-DARPin and small-molecule binders identified through fragment screening, remains an active area of preclinical investigation.^{49,52} Preclinical evidence establishes SMO-dependent SHH-Gli1 signaling as a key driver in chordoma, with high Gli1 expression predicting poor prognosis. The SMO inhibitor vismodegib demonstrated significant antitumor activity in chordoma cell lines and xenograft models, validating Shh as a therapeutic target complementary to Brachyury. Given that direct Brachyury inhibitors are still in early development, these data support advancing hedgehog inhibitors into clinical trials, underscoring the necessity of biomarker-driven approaches for future regulatory approval.^{95,96} Overall, no targeted therapy has yet received regulatory approval specifically for chordoma, highlighting the need for continued drug development and biomarker-driven clinical trials.

Immunotherapy: remodeling the tumor microenvironment. Immunotherapeutic strategies have been explored in chordoma, but clinical translation remains limited. For example, active immunotherapy targeting Brachyury was evaluated in a phase I dose-escalation trial (n = 13) of patients with advanced solid tumors, including chordoma. The vaccine, administered intravenously as MVA-BN-Brachyury-TRICOM, was well tolerated and elicited Brachyury-specific T-cell responses. Among 13 evaluable patients, one RECIST PR was observed.⁹⁷ A subsequent phase II trial evaluated a different subcutaneous “prime-boost” BN-Brachyury vaccine regimen combined with radiation therapy. Among 26 evaluable chordoma patients, the trial reported two mRECIST PRs.⁵¹

T cells are central to immune checkpoint inhibitor (ICI) therapy. In chordoma, the PD-1/PD-L1 axis has been a major research focus. Clinical evidence supporting its relevance includes immunohistochemical analysis of 78 chordoma specimens, which showed that 94.9% were PD-L1 positive,⁶⁶ and this expression significantly correlated with tumor-infiltrating lymphocyte abundance and metastasis. These findings provide a rationale for clinical investigation.

Accordingly, a case report described favorable antitumor responses to the anti-PD-1 antibody pembrolizumab in chordoma patients.⁹⁸ However, robust evidence from larger prospective trials is still lacking. Importantly, due to tumor heterogeneity and other immune resistance mechanisms, PD-L1 expression alone is not a reliable predictor of response to ICI in chordoma. CAR-T therapy involves modifying T cells with chimeric antigen receptors to specifically recognize tumor-associated antigens. Single-cell RNA-seq of 14 chordoma samples identified chordoma-specific antigens (e.g., VEGFR) and TGF- β signaling as potential targets for CAR-T cell therapy, potentially enhancing the efficacy of CAR-T cells.⁹⁹ Beyond VEGFR, B7-H3 represents another promising CAR-T target, characterized by universal high expression in chordoma tissues and limited expression in normal tissues.¹⁰⁰ Preclinical studies in xenograft models suggest that combining B7-H3 CAR-T cells with radiotherapy can significantly improve antitumor efficacy.¹⁰⁰ Moreover, engineering the B7-H3 CAR to include IL-7 may further augment CAR-T cell function and potency, offering a novel dual-therapy approach for chordoma.¹⁰¹ Despite these promising preclinical insights, no clinical trials of CAR-T therapy for chordoma have been reported to date.

In summary, although immunotherapy holds promise for chordoma, no immunotherapeutic agent has yet received regulatory approval for this indication. Future efforts should focus on combination strategies (e.g., vaccine combined with ICI or CAR-T plus radiotherapy) and the identification of predictive biomarkers.

Precision subtyping therapy: individualized strategies based on molecular subtypes. Despite advances in chordoma therapy, tumor heterogeneity results in variable clinical outcomes, underscoring the need for personalized approaches. The current histological classification (conventional, dedifferentiated, poorly differentiated) provides prognostic value but remains too broad to guide precise therapeutic decisions.⁸⁹

Transcriptomic profiling has identified two distinct molecular subtypes (CC1 and CC2) of chordoma, with the CC2 subtype showing upregulation of EMT and SHH signaling pathways, while CC1 is characterized by chromatin remodeling gene mutations.¹⁰² The association between SHH pathway activation and the CC2 subtype is noteworthy from a developmental biology perspective. During embryonic development, Shh acts as a morphogen whose concentration and exposure duration determine distinct cell fates.¹⁴⁻¹⁶ In chordoma, aberrant

reactivation of this developmental pathway in the CC2 subtype may drive a more aggressive transcriptional program. Consistent with this notion, the CC2 subtype exhibits a more invasive phenotype compared with CC1 tumors.¹⁰² This developmental parallelism suggests that SHH pathway activation is not merely a molecular marker but may function as a bona fide driver of subtype-specific biology, raising the possibility that SHH inhibitors could be therapeutically effective in CC2-enriched patient populations. Furthermore, DNA methylation profiling has identified two distinct epigenetic subtypes (C and I) of skull base chordomas: subtype C is characterized by global hypomethylation with CpG island hypermethylation, upregulation of cell cycle, and higher chromosomal instability, while subtype I shows global hypermethylation and an immune-enriched microenvironment.⁵⁸ Subsequently, a proteomic analysis involving 102 chordoma patients was conducted to classify chordomas into distinct molecular subtypes and to propose precise treatment strategies based on these subtypes.¹⁰³ Non-negative matrix factorization (NMF) clustering revealed three distinct molecular subtypes. The first, a bone microenvironment (BM)-dominant subtype, was strongly associated with osteoclastogenesis and immunogenicity. The second, a mesenchymal-derived subtype, exhibited high activity in mitochondrial oxidative phosphorylation (OXPHOS). The third, a mesenchymal-to-epithelial transition (MET)-mediated subtype, showed a strong association with activated RTK signaling, particularly involving the FGFR and PI3K-AKT pathways. Based on these distinct patterns, Denosumab, S-Gboxin, and Anlotinib were selected as targeted options, each exerting marked suppressive effects. To facilitate preclinical validation of these subtype-specific therapies, a subsequent study established corresponding *in vivo* and *in vitro* models, including cell-derived and patient-derived xenografts, based on these proteomic subtypes.¹⁰⁴

However, the implementation of proteomic subtyping in routine clinical practice remains challenging due to high costs, limited tissue availability, and the need for prospective validation in large, multicenter cohorts (see Table 1 for a summary of treatment strategies).^{105,106} Table 2 compares notochord cell fate across five dimensions (definition, cellular origin, stage of occurrence, key drivers, and microenvironmental features) between normal differentiation (NP formation) and malignant transformation (chordoma development). This comparison illustrates the molecular basis and translational value of distinct cell fates from the perspective of the “shared notochord lineage”.

Challenges in clinical translation

Despite the promising therapeutic strategies described above, several barriers impede their translation into clinical practice.

Safety concerns. On-target off-tumor toxicities, particularly for Brachyury-targeted and CAR-T therapies, remain poorly characterized in chordoma. Clinical data for these approaches are extremely limited: no phase III trials of a Brachyury-targeted agent have been completed in chordoma, and no CAR-T clinical trial has been initiated to date.¹⁰⁷ Additionally, while YAP inhibitors are attractive in principle, they may disrupt normal tissue homeostasis given the essential roles of Hippo signaling in development and adult physiology.⁵³ Collectively, these safety uncertainties, combined with the lack of clinical data and predictive tools, render the direct clinical application of Brachyury-targeted and CAR-T therapies challenging at present. Addressing these obstacles will require international consortia, standardized biobanking, and adaptive clinical trial designs tailored to rare diseases.

Tumor heterogeneity. Single-cell RNA-seq has identified six functionally distinct chordoma cell subclusters, directly demonstrating intratumoral heterogeneity.⁶⁵ Moreover, transcriptomic profiles differ between skull base and spinal chordomas, indicating interpatient heterogeneity.¹⁰⁸ Consequently, a single biopsy may not capture the full molecular landscape, and repeat or multi-region sampling may be required to guide precision therapy.¹⁰⁹

Delivery challenges. The treatment of chordoma faces dual challenges. On the one hand, its unique physical microenvironment represents a significant biological barrier. The dense ECM, rich in collagen and proteoglycans, together with the highly sclerotic bone microenvironment, severely restricts the diffusion and penetration of small-molecule targeted drugs. It also hinders the infiltration and survival of adoptive cell therapies, such as CAR-T cells, rendering many therapies that are effective in other tumors less efficacious in chordoma.^{107,110} On the other hand, the epidemiological characteristics of the disease pose challenges for clinical research. Because chordoma is an extremely rare tumor with a low annual incidence, the small patient pool makes it exceptionally difficult to recruit enough participants for large-scale, randomized controlled phase III clinical trials. This, in turn, hampers the generation of high-level medical data and slows the accumulation of long-term safety and efficacy assessments for new therapies.^{111,112}

FUTURE RESEARCH AND CHALLENGES

From the perspective of the “shared notochord lineage”, this review systematically delineates the fate trajectory of the notochord from embryonic development to NP formation and tumorigenesis. This framework not only elucidates the regulatory networks of notochord cells across physiological and pathological states but also provides a roadmap for future research and treatment. Despite significant progress in recent years, substantial challenges remain. Breakthroughs are urgently needed in three key areas: mechanistic understanding, model development, and interdisciplinary integration.

Mechanistic understanding: from molecular networks to spatiotemporal integration

Current understanding of the regulatory mechanisms underlying notochord development and fate determination has advanced considerably, but several key questions remain. Quantitative analysis of mechanical force signals is one important direction. AFM has been used to measure the stiffness gradient along the dorsoventral axis of the developing neural tube and to identify YAP as a mechanotransducer. However, how mechanical forces are precisely sensed during notochord morphogenesis, and how they cooperate with biochemical signals (such as WNT and TGF- β) to regulate cell fate, remain mechanistically unclear. Future efforts should combine *in vivo* mechanical measurements with single-cell resolution imaging to establish dynamic maps of mechanosignal transduction.

Another key issue is the temporal control of signaling pathways. Rito *et al.*¹² found that the “time window” of TGF- β signaling determines notochord fate: delaying TGF- β inhibition by 24 hours markedly increases the proportion of notochord-like cells, whereas inhibiting too early or too late directs cells toward other fates. This finding suggests that the activation and inhibition of signaling pathways during development are not simple on-off switches, but rather dynamic processes governed by precise timing. Future work should further dissect the molecular basis of “time windows”, their conservation across species, and their potential dysregulation in chordoma.

Model development: from 2D cultures to 3D organoids

Traditional research models have inherent limitations in dissecting notochord biology. Two-dimensional (2D) cell cultures fail to recapitulate the 3D architecture and mechanical

microenvironment of the notochord, whereas animal models are constrained by interspecies differences and technical complexity. In recent years, rapid advances in organoid technology have provided a new platform for notochord research. A 3D trunk organoid model containing the notochord, neural tube, and somites was successfully established through precise temporal control of signaling pathways in human pluripotent stem cells.¹² This model not only recapitulates notochord morphogenesis and cellular features such as vacuoles, basement membrane, and extracellular matrix sheath but also demonstrates the potential inductive role of the notochord on surrounding neural tissues.

For chordoma, although molecular subtyping (e.g., bone microenvironment-dominant, mesenchymal-derived, and MET-mediated) provides a framework for precision therapy,¹⁰³ substantial validation is still needed to translate these subtypes into clinically actionable strategies. Organoid and PDX models can serve as touchstones for drug screening, accelerating the clinical translation of potential targets such as CA2, mTOR, and TGF- β . However, no single model system is perfect: PDX models lack a functional human immune system;¹¹³ zebrafish models may not fully recapitulate human chordoma genetics;¹¹⁴ long-term culture of chordoma cell lines can lead to phenotypic drift,¹¹⁵ and organoids lack immune and vascular components.⁷⁷ Future efforts should focus on integrating multiple complementary models, including patient-derived organoids, humanized PDX models, and genetically engineered mouse models, to rigorously validate therapeutic candidates.

Interdisciplinary integration: driving a paradigm shift in research

Research on notochord biology is shifting from a traditional molecular biology paradigm toward a multidisciplinary approach integrating biomechanics, organoid engineering, single-cell omics, and artificial intelligence (AI). Single-cell and spatial transcriptomics have already revealed cellular heterogeneity during notochord development and chordoma. Future integration with spatial transcriptomics will further elucidate the spatiotemporal dynamics of cell-cell interactions within the notochord microenvironment. The convergence of biomechanics and tissue engineering will drive a shift from “static culture” to “dynamic simulation”, providing more physiologically relevant *in vitro* models to reveal the role of mechanical forces in notochord fate determination. Meanwhile, AI applications in image

analysis, drug screening, and predictive modeling will accelerate the translation from basic discoveries to clinical practice.¹¹⁶

CONCLUSION

This review introduces a “shared notochord lineage” framework that unifies notochord development, NP formation, and chordoma tumorigenesis. The notochord gives rise to two major postnatal branches: the NP (the normal fate) and notochordal remnants that, under rare genetic and microenvironmental conditions, may undergo malignant transformation into chordoma. By systematically comparing the regulatory networks governing notochord development, including mechanotransduction via YAP signaling, Shh concentration-time gradients, and Brachyury-driven transcription, with their aberrant reactivation in chordoma, we demonstrate how developmental pathways are co-opted to drive tumor stemness, therapy resistance, and disease progression.

YAP plays stage- and context-dependent roles: its inactivation is required for early notochord fate specification, whereas its activation is essential for later mechanotransduction and Shh expression. This same YAP-Brachyury axis is hijacked in chordoma to sustain tumor stemness. Molecular subtyping, including transcriptomic CC1/CC2 and proteomic subtypes (BM-dominant, mesenchymal-derived, MET-mediated) provides a roadmap for precision oncology. Emerging therapies, such as Brachyury-targeted vaccines, YAP inhibitors, CAR-T cells, and subtype-guided agents (denosumab, S-Gboxin, anlotinib), are beginning to translate developmental biology into clinical testing, although none has yet received regulatory approval specifically for chordoma.

Several obstacles remain. The mechanisms driving benign-to-malignant transformation of notochordal remnants remain poorly understood. The dense ECM and sclerotic bone microenvironment severely impede the delivery of both small-molecule drugs and cell-based therapies. Moreover, the rarity of chordoma makes large-scale randomized trials challenging, and most evidence currently remains at the preclinical or early clinical stage. To address these challenges, international consortia and adaptive trial designs tailored to rare cancers are essential. Multi-region and longitudinal biopsies, combined with single-cell and spatial omics, should be implemented to capture intratumoral heterogeneity. At the preclinical level, the integration of organoid, PDX, and humanized mouse models will accelerate the validation of

novel targets such as CA2, YAP, and Brachyury. At the clinical level, combination strategies (e.g., vaccine plus immune checkpoint blockade, CAR-T plus radiotherapy, and YAP inhibitor plus conventional therapy) offer the greatest promise.

Unlike previous reviews that treated development, degeneration, and tumorigenesis as separate topics, our “shared notochord lineage” framework explicitly links developmental pathways to chordoma biology. This shifts the research paradigm from a descriptive account of chordoma genetics toward a mechanism-driven, development-informed approach. It encourages researchers to ask which other developmental regulators are reactivated in chordoma, and whether the temporal dynamics of signaling pathways (e.g., the TGF- β window) can be exploited therapeutically. Answering these questions will not only deepen our understanding of chordoma but also accelerate the translation of developmental biology discoveries into clinical practice.

REFERENCES

1. Zhou T., Chen Y., Liao Z., et al. (2023). Spatiotemporal characterization of human early intervertebral disc formation at single-cell resolution. *Adv. Sci. (Weinh)* **10**:e2206296. DOI:10.1002/advs.202206296
2. Harfe B. D. (2022). Intervertebral disc repair and regeneration: Insights from the notochord. *Semin. Cell Dev. Biol.* **127**:3-9. DOI:10.1016/j.semcdb.2021.11.012
3. Choi K. S., Cohn M. J. and Harfe B. D. (2008). Identification of nucleus pulposus precursor cells and notochordal remnants in the mouse: Implications for disk degeneration and chordoma formation. *Dev. Dyn.* **237**:3953-3958. DOI:10.1002/dvdy.21805
4. Yamaguchi T., Suzuki S., Ishiwa H., et al. (2004). Intraosseous benign notochordal cell tumours: Overlooked precursors of classic chordomas?

- Histopathology* **44**:597-602. DOI:10.1111/j.1365-2559.2004.01877.x
5. Ramesh T., Nagula S. V., Tardieu G. G., et al. (2017). Update on the notochord including its embryology, molecular development, and pathology: A primer for the clinician. *Cureus* **9**:e1137. DOI:10.7759/cureus.1137
 6. Corallo D., Trapani V. and Bonaldo P. (2015). The notochord: Structure and functions. *Cell. Mol. Life Sci.* **72**:2989-3008. DOI:10.1007/s00018-015-1897-z
 7. Sun X., Hornicek F. and Schwab J. H. (2015). Chordoma: An update on the pathophysiology and molecular mechanisms. *Curr. Rev. Musculoskelet. Med.* **8**:344-352. DOI:10.1007/s12178-015-9311-x
 8. Yamamoto M., Morita R., Mizoguchi T., et al. (2010). Mib-jag1-notch signalling regulates patterning and structural roles of the notochord by controlling cell-fate decisions. *Development* **137**:2527-2537. DOI:10.1242/dev.051011
 9. Sánchez-Iranzo H., Halavatyi A. and Diz-Muñoz A. (2022). Strength of interactions in the notch gene regulatory network determines patterning and fate in the notochord. *Elife* **11**. DOI:10.7554/eLife.75429
 10. Bagnat M., Daga B. and Di Talia S. (2022). Morphogenetic roles of hydrostatic pressure in animal development. *Annu. Rev. Cell Dev. Biol.* **38**:375-394. DOI:10.1146/annurev-cellbio-120320-033250
 11. Cheng C., Cong Q., Liu Y., et al. (2023). Yap controls notochord formation and neural tube patterning by integrating mechanotransduction with *foxa2* and *shh* expression. *Sci. Adv.* **9**:eadf6927. DOI:10.1126/sciadv.adf6927

12. Rito T., Libby A. R. G., Demuth M., et al. (2025). Timely $\text{tgf}\beta$ signalling inhibition induces notochord. *Nature* **637**:673-682. DOI:10.1038/s41586-024-08332-w

13. Shah S. R., David J. M., Tippens N. D., et al. (2017). Brachyury-yap regulatory axis drives stemness and growth in cancer. *Cell Rep.* **21**:495-507. DOI:10.1016/j.celrep.2017.09.057

14. Ericson J., Muhr J., Placzek M., et al. (1995). Sonic hedgehog induces the differentiation of ventral forebrain neurons: A common signal for ventral patterning within the neural tube. *Cell* **81**:747-756. DOI:10.1016/0092-8674(95)90536-7

15. Yamada T., Placzek M., Tanaka H., et al. (1991). Control of cell pattern in the developing nervous system: Polarizing activity of the floor plate and notochord. *Cell* **64**:635-647. DOI:10.1016/0092-8674(91)90247-v

16. Dessaud E., Yang L. L., Hill K., et al. (2007). Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism. *Nature* **450**:717-720. DOI:10.1038/nature06347

17. Makwana K., Tilley L., Chakravarty P., et al. (2025). Modelling co-development between the somites and neural tube in human trunk-like structures. *Nat. Cell Biol.* **27**:2049-2062. DOI:10.1038/s41556-025-01813-8

18. Choi K. S. and Harfe B. D. (2011). Hedgehog signaling is required for formation of the notochord sheath and patterning of nuclei pulposi within the intervertebral discs. *Proc. Natl. Acad. Sci. U S A* **108**:9484-9489.

- DOI:10.1073/pnas.1007566108
19. Maier J. A., Lo Y. and Harfe B. D. (2013). *Foxa1* and *foxa2* are required for formation of the intervertebral discs. *PLoS One* **8**:e55528.
- DOI:10.1371/journal.pone.0055528
20. Wilkinson D. G., Bhatt S. and Herrmann B. G. (1990). Expression pattern of the mouse *t* gene and its role in mesoderm formation. *Nature* **343**:657-659.
- DOI:10.1038/343657a0
21. Kemmler C. L., Smolikova J., Moran H. R., et al. (2023). Conserved enhancers control notochord expression of vertebrate brachyury. *Nat. Commun.* **14**:6594. DOI:10.1038/s41467-023-42151-3
22. Schifferl D., Scholze-Wittler M., Wittler L., et al. (2021). A 37 kb region upstream of brachyury comprising a notochord enhancer is essential for notochord and tail development. *Development* **148**. DOI:10.1242/dev.200059
23. McCann M. R., Tamplin O. J., Rossant J., et al. (2012). Tracing notochord-derived cells using a noto-cre mouse: Implications for intervertebral disc development. *Dis. Model. Mech.* **5**:73-82. DOI:10.1242/dmm.008128
24. Karele E. N. and Paze A. N. (2022). Chordoma: To know means to recognize. *Biochim Biophys Acta Rev Cancer* **1877**:188796.
- DOI:10.1016/j.bbcan.2022.188796
25. Aszódi A., Chan D., Hunziker E., et al. (1998). Collagen ii is essential for the removal of the notochord and the formation of intervertebral discs. *J. Cell Biol.* **143**:1399-1412. DOI:10.1083/jcb.143.5.1399

26. Lawson L. and Harfe B. D. (2015). Notochord to nucleus pulposus transition. *Curr. Osteoporos. Rep.* **13**:336-341. DOI:10.1007/s11914-015-0284-x

27. Peck S. H., McKee K. K., Tobias J. W., et al. (2017). Whole transcriptome analysis of notochord-derived cells during embryonic formation of the nucleus pulposus. *Sci. Rep.* **7**:10504. DOI:10.1038/s41598-017-10692-5

28. Zhang C., Zhong L., Lau Y. K., et al. (2024). Single cell rna sequencing reveals emergent notochord-derived cell subpopulations in the postnatal nucleus pulposus. *FASEB J.* **38**:e23363. DOI:10.1096/fj.202301217R

29. Tan Z., Chen P., Dong X., et al. (2024). Progenitor-like cells contributing to cellular heterogeneity in the nucleus pulposus are lost in intervertebral disc degeneration. *Cell Rep.* **43**:114342. DOI:10.1016/j.celrep.2024.114342

30. Chen Y., Zhang L., Shi X., et al. (2024). Characterization of the nucleus pulposus progenitor cells via spatial transcriptomics. *Adv. Sci. (Weinh)* **11**:e2303752. DOI:10.1002/advs.202303752

31. Merceron C., Mangiavini L., Robling A., et al. (2014). Loss of hif-1 α in the notochord results in cell death and complete disappearance of the nucleus pulposus. *PLoS One* **9**:e110768. DOI:10.1371/journal.pone.0110768

32. Alvarez-Garcia O., Matsuzaki T., Olmer M., et al. (2018). Foxo are required for intervertebral disk homeostasis during aging and their deficiency promotes disk degeneration. *Aging Cell* **17**:e12800. DOI:10.1111/acer.12800

33. Wang L., Jin K., Li N., et al. (2023). Innovative design of minimal invasive biodegradable poly(glycerol-dodecanoate) nucleus pulposus scaffold with

- function regeneration. *Nat. Commun.* **14**:3865. DOI:10.1038/s41467-023-39604-0
34. Hunter C. J., Matyas J. R. and Duncan N. A. (2003). The notochordal cell in the nucleus pulposus: A review in the context of tissue engineering. *Tissue Eng.* **9**:667-677. DOI:10.1089/107632703768247368
35. McCann M. R. and Séguin C. A. (2016). Notochord cells in intervertebral disc development and degeneration. *J. Dev. Biol.* **4**. DOI:10.3390/jdb4010003
36. Erwin W. M., Islam D., Inman R. D., et al. (2011). Notochordal cells protect nucleus pulposus cells from degradation and apoptosis: Implications for the mechanisms of intervertebral disc degeneration. *Arthritis Res. Ther.* **13**:R215. DOI:10.1186/ar3548
37. Mohd Isa I. L., Teoh S. L., Mohd Nor N. H., et al. (2022). Discogenic low back pain: Anatomy, pathophysiology and treatments of intervertebral disc degeneration. *Int. J. Mol. Sci.* **24**. DOI:10.3390/ijms24010208
38. Zhou M., Theologis A. A. and O'Connell G. D. (2024). Understanding the etiopathogenesis of lumbar intervertebral disc herniation: From clinical evidence to basic scientific research. *JOR Spine* **7**:e1289. DOI:10.1002/jsp2.1289
39. Zhang A. S., Xu A., Ansari K., et al. (2023). Lumbar disc herniation: Diagnosis and management. *Am. J. Med.* **136**:645-651. DOI:10.1016/j.amjmed.2023.03.024
40. Orozco L., Soler R., Morera C., et al. (2011). Intervertebral disc repair by

autologous mesenchymal bone marrow cells: A pilot study. *Transplantation* **92**:822-828. DOI:10.1097/TP.0b013e3182298a15

41. Mochida J., Sakai D., Nakamura Y., et al. (2015). Intervertebral disc repair with activated nucleus pulposus cell transplantation: A three-year, prospective clinical study of its safety. *Eur Cell Mater* **29**:202-212; discussion 212. DOI:10.22203/ecm.v029a15

42. Wang H., Xu A., Hua W., et al. (2025). Stem cell-based strategies for intervertebral disc regeneration in degenerative microenvironments: Challenges and solutions. *Front. Cell Dev. Biol.* **13**:1719332. DOI:10.3389/fcell.2025.1719332

43. Su Z., Dong H., Fang X., et al. (2025). Frontier progress and translational challenges of pluripotent differentiation of stem cells. *Front. Genet.* **16**:1583391. DOI:10.3389/fgene.2025.1583391

44. Gradisnik L., Prestor B., Maver U., et al. (2026). From bench to bedside: Translational barriers in mesenchymal stem cell therapy for intervertebral disc degeneration. *Bioengineering (Basel)* **13**. DOI:10.3390/bioengineering13050544

45. Meng T., Huang R., Jin J., et al. (2021). A comparative integrated multi-omics analysis identifies ca2 as a novel target for chordoma. *Neuro. Oncol.* **23**:1709-1722. DOI:10.1093/neuonc/noab156

46. Mendenhall W. M., Mendenhall C. M., Lewis S. B., et al. (2005). Skull base chordoma. *Head Neck* **27**:159-165. DOI:10.1002/hed.20144

- 1
2
3
4 47. Li M., Shen Y., Xiong Y., et al. (2021). Loss of smarcb1 promotes autophagy
5
6 and facilitates tumour progression in chordoma by transcriptionally activating
7
8 atg5. *Cell Prolif.* **54**:e13136. DOI:10.1111/cpr.13136
9
10
11 48. Hu Y., Mintz A., Shah S. R., et al. (2014). The fgfr/mek/erk/brachyury pathway
12
13 is critical for chordoma cell growth and survival. *Carcinogenesis* **35**:1491-
14
15 1499. DOI:10.1093/carcin/bgu014
16
17
18 49. Newman J. A., Gavard A. E., Imprachim N., et al. (2025). Structural insights
19
20 into human brachyury DNA recognition and discovery of progressible binders
21
22 for cancer therapy. *Nat. Commun.* **16**:1596. DOI:10.1038/s41467-025-56213-
23
24 1
25
26
27 50. Chase D. H., Bebenek A. M., Nie P., et al. (2024). Development of a small
28
29 molecule downmodulator for the transcription factor brachyury. *Angew.*
30
31 *Chem. Int. Ed. Engl.* **63**:e202316496. DOI:10.1002/anie.202316496
32
33
34 51. Cote G. M., Conley A. P., Attia S., et al. (2024). A phase 2 study of a
35
36 brachyury-targeting vaccine in combination with radiation therapy for the
37
38 treatment of advanced chordoma. *Cancer* **130**:3845-3854.
39
40
41 DOI:10.1002/cncr.35477
42
43
44 52. Umbaugh C. S., Groth M., Erkut C., et al. (2025). Selective targeting of tbxt
45
46 with darpins identifies regulatory networks and therapeutic vulnerabilities in
47
48 chordoma. *Sci. Adv.* **11**:eadu2796. DOI:10.1126/sciadv.adu2796
49
50
51 53. Kakiuchi-Kiyota S., Schutten M. M., Zhong Y., et al. (2019). Safety
52
53 considerations in the development of hippo pathway inhibitors in cancers.
54
55
56
57
58
59
60

- Front. Cell Dev. Biol.* **7**:156. DOI:10.3389/fcell.2019.00156
54. Baroja I., Kyriakidis N. C., Halder G., et al. (2024). Expected and unexpected effects after systemic inhibition of hippo transcriptional output in cancer. *Nat. Commun.* **15**:2700. DOI:10.1038/s41467-024-46531-1
55. Hsia B., Bitar G., Alshaka S. A., et al. (2025). Genomic characterization of chordoma: Insights from the aacr project genie database. *Cancers (Basel)* **17**. DOI:10.3390/cancers17030536
56. Walhart T. A., Vacca B., Hepperla A. J., et al. (2023). Smarcb1 loss in poorly differentiated chordomas drives tumor progression. *Am. J. Pathol.* **193**:456-473. DOI:10.1016/j.ajpath.2022.12.012
57. Beisaw A., Tsaytler P., Koch F., et al. (2018). Brachyury directs histone acetylation to target loci during mesoderm development. *EMBO Rep.* **19**:118-134. DOI:10.15252/embr.201744201
58. Baluszek S., Kober P., Rusetska N., et al. (2023). DNA methylation, combined with rna sequencing, provide novel insight into molecular classification of chordomas and their microenvironment. *Acta Neuropathol. Commun.* **11**:113. DOI:10.1186/s40478-023-01610-0
59. Bai J., Shi J., Li C., et al. (2021). Whole genome sequencing of skull-base chordoma reveals genomic alterations associated with recurrence and chordoma-specific survival. *Nat. Commun.* **12**:757. DOI:10.1038/s41467-021-21026-5
60. Wang L., Zehir A., Nafa K., et al. (2016). Genomic aberrations frequently alter

- chromatin regulatory genes in chordoma. *Genes Chromosomes Cancer* **55**:591-600. DOI:10.1002/gcc.22362
61. Le L. P., Nielsen G. P., Rosenberg A. E., et al. (2011). Recurrent chromosomal copy number alterations in sporadic chordomas. *PLoS One* **6**:e18846. DOI:10.1371/journal.pone.0018846
 62. Zhang Q., Xu Z., Han R., et al. (2024). Proteogenomic characterization of skull-base chordoma. *Nat. Commun.* **15**:8338. DOI:10.1038/s41467-024-52285-7
 63. Baluszek S., Kober P., Wagrodzki M., et al. (2025). Tp53 mutations as drivers of chordoma progression and hallmarks of aggressive chordoma. *Acta Neuropathol. Commun.* **14**:24. DOI:10.1186/s40478-025-02180-z
 64. Chen H., Garbutt C. C., Spentzos D., et al. (2017). Expression and therapeutic potential of sox9 in chordoma. *Clin. Cancer Res.* **23**:5176-5186. DOI:10.1158/1078-0432.Ccr-17-0177
 65. Zhang Q., Fei L., Han R., et al. (2022). Single-cell transcriptome reveals cellular hierarchies and guides p-emt-targeted trial in skull base chordoma. *Cell Discov.* **8**:94. DOI:10.1038/s41421-022-00459-2
 66. Feng Y., Shen J., Gao Y., et al. (2015). Expression of programmed cell death ligand 1 (pd-l1) and prevalence of tumor-infiltrating lymphocytes (tils) in chordoma. *Oncotarget* **6**:11139-11149. DOI:10.18632/oncotarget.3576
 67. Zhang H., Xing J., Yuan Z., et al. (2026). Targeting the tumor immune microenvironment in chordoma: From mechanistic insights to therapeutic

breakthroughs. *Biochim. Biophys. Acta Rev. Cancer* **1881**:189520.

DOI:10.1016/j.bbcan.2025.189520

68. Chen Y. and Zhang H. (2024). Immune microenvironment and immunotherapy for chordoma. *Front. Oncol.* **14**:1374249.

DOI:10.3389/fonc.2024.1374249

69. Xu J., Shi Q., Lou J., et al. (2023). Chordoma recruits and polarizes tumor-associated macrophages via secreting ccl5 to promote malignant progression. *J. Immunother. Cancer* **11**. DOI:10.1136/jitc-2023-006808

70. Fujii R., Friedman E. R., Richards J., et al. (2016). Enhanced killing of chordoma cells by antibody-dependent cell-mediated cytotoxicity employing the novel anti-pd-l1 antibody avelumab. *Oncotarget* **7**:33498-33511.

DOI:10.18632/oncotarget.9256

71. Liang S., Luo B., Xie S., et al. (2025). The immune microenvironment in chordoma: Implications for future treatment. *World Neurosurg.* **204**:124589.

DOI:10.1016/j.wneu.2025.124589

72. Walker R. L., Hornicek F. J. and Duan Z. (2022). Advances in the development of chordoma models for drug discovery and precision medicine. *Biochim. Biophys. Acta Rev. Cancer* **1877**:188812.

DOI:10.1016/j.bbcan.2022.188812

73. Ravi M., Paramesh V., Kaviya S. R., et al. (2015). 3d cell culture systems: Advantages and applications. *J. Cell. Physiol.* **230**:16-26.

DOI:10.1002/jcp.24683

- 1
2
3
4 74. Davies J. M., Robinson A. E., Cowdrey C., et al. (2014). Generation of a
5
6 patient-derived chordoma xenograft and characterization of the
7
8 phosphoproteome in a recurrent chordoma. *J. Neurosurg.* **120**:331-336.
9
10 DOI:10.3171/2013.10.Jns13598
11
12
13
14 75. Burger A., Vasilyev A., Tomar R., et al. (2014). A zebrafish model of
15
16 chordoma initiated by notochord-driven expression of hrasv12. *Dis. Model*
17
18 *Mech.* **7**:907-913. DOI:10.1242/dmm.013128
19
20
21
22 76. LeSavage B. L., Suhar R. A., Broguiere N., et al. (2022). Next-generation
23
24 cancer organoids. *Nat. Mater.* **21**:143-159. DOI:10.1038/s41563-021-01057-5
25
26
27
28 77. Yuki K., Cheng N., Nakano M., et al. (2020). Organoid models of tumor
29
30 immunology. *Trends Immunol.* **41**:652-664. DOI:10.1016/j.it.2020.06.010
31
32
33 78. Wolff L. and Hendrix S. (2025). Rethinking matrigel: The complex journey to
34
35 matrix alternatives in organoid culture. *Adv. Sci. (Weinh)* **12**:e08734.
36
37 DOI:10.1002/advs.202508734
38
39
40 79. Ma Z., Liu Y., Chen R., et al. (2025). A novel perspective on bone tumors:
41
42 Advances in organoid research. *Front. Pharmacol.* **16**:1550163.
43
44 DOI:10.3389/fphar.2025.1550163
45
46
47
48 80. Stacchiotti S. and Sommer J. (2015). Building a global consensus approach
49
50 to chordoma: A position paper from the medical and patient community.
51
52 *Lancet Oncol.* **16**:e71-83. DOI:10.1016/s1470-2045(14)71190-8
53
54
55
56 81. Baumann B. C., Lustig R. A., Mazzoni S., et al. (2019). A prospective clinical
57
58 trial of proton therapy for chordoma and chondrosarcoma: Feasibility
59
60

- assessment. *J. Surg. Oncol.* **120**:200-205. DOI:10.1002/jso.25502
82. Imai R., Kamada T. and Araki N. (2016). Carbon ion radiation therapy for unresectable sacral chordoma: An analysis of 188 cases. *Int. J. Radiat. Oncol. Biol. Phys.* **95**:322-327. DOI:10.1016/j.ijrobp.2016.02.012
83. Bin Sumaida A., Shanbhag N. M., AlKaabi K., et al. (2025). Cost-effectiveness of carbon ion radiotherapy in oncology: A systematic review. *Cureus* **17**:e84008. DOI:10.7759/cureus.84008
84. Dong M., Liu R., Zhang Q., et al. (2023). Efficacy and safety of carbon ion radiotherapy for chordomas: A systematic review and meta-analysis. *Radiat. Oncol.* **18**:152. DOI:10.1186/s13014-023-02337-x
85. Seidensaal K., Froehlke A., Lentz-Hommertgen A., et al. (2024). Hypofractionated proton and carbon ion beam radiotherapy for sacrococcygeal chordoma (isac): An open label, randomized, stratified, phase ii trial. *Radiother. Oncol.* **198**:110418. DOI:10.1016/j.radonc.2024.110418
86. Tamborini E., Virdis E., Negri T., et al. (2010). Analysis of receptor tyrosine kinases (rtks) and downstream pathways in chordomas. *Neuro Oncol.* **12**:776-789. DOI:10.1093/neuonc/noq003
87. Barber S. M., Sadrameli S. S., Lee J. J., et al. (2021). Chordoma-current understanding and modern treatment paradigms. *J. Clin. Med.* **10**. DOI:10.3390/jcm10051054
88. Hu P., Lei J., Du S., et al. (2026). Immune and stromal signaling networks in chordoma: An evidence-weighted review of ligand-receptor interactions and

- therapeutic implications. *Syst. Rev.* **15**. DOI:10.1186/s13643-026-03183-0
89. Desai R., Pelargos P. E. and Dunn I. F. (2024). Chordoma: Genetics and contemporary management. *Int. J. Mol. Sci.* **25**. DOI:10.3390/ijms25115877
90. Liu C., Jia Q., Wei H., et al. (2020). Apatinib in patients with advanced chordoma: A single-arm, single-centre, phase 2 study. *Lancet Oncol.* **21**:1244-1252. DOI:10.1016/s1470-2045(20)30466-6
91. Yang C., Jia Q., Zhao C., et al. (2026). Efficacy and safety of camrelizumab plus apatinib in patients with refractory chordoma: A phase ii clinical trial. *J. Clin. Oncol.*:Jco2502719. DOI:10.1200/jco-25-02719
92. Stacchiotti S., Tamborini E., Lo Vullo S., et al. (2013). Phase ii study on lapatinib in advanced egfr-positive chordoma. *Ann. Oncol.* **24**:1931-1936. DOI:10.1093/annonc/mdt117
93. Meng T., Jin J., Jiang C., et al. (2019). Molecular targeted therapy in the treatment of chordoma: A systematic review. *Front. Oncol.* **9**:30. DOI:10.3389/fonc.2019.00030
94. Teleanu M. V., Heilig C. E., Pirmann S., et al. (2025). Cdk4/6 inhibition in advanced chordoma: Final results of the nct pmo-1601 trial. *ESMO Open* **10**:105498. DOI:10.1016/j.esmoop.2025.105498
95. Akhavan-Sigari R., Schulz-Schaeffer W., Angelika Harcej A., et al. (2019). The importance of the hedgehog signaling pathway in tumorigenesis of spinal and cranial chordoma. *J. Clin. Med.* **8**. DOI:10.3390/jcm8020248
96. Yang C., Yong L., Liang C., et al. (2020). Genetic landscape and ligand-

dependent activation of sonic hedgehog-gli1 signaling in chordomas: A novel therapeutic target. *Oncogene* **39**:4711-4727. DOI:10.1038/s41388-020-1324-2

97. DeMaria P. J., Lee-Wisdom K., Donahue R. N., et al. (2021). Phase 1 open-label trial of intravenous administration of mva-bn-brachyury-tricom vaccine in patients with advanced cancer. *J. Immunother. Cancer* **9**. DOI:10.1136/jitc-2021-003238

98. Wu X., Lin X., Chen Y., et al. (2020). Response of metastatic chordoma to the immune checkpoint inhibitor pembrolizumab: A case report. *Front. Oncol.* **10**:565945. DOI:10.3389/fonc.2020.565945

99. Wu H., Li X., Zhang B., et al. (2024). Single-cell sequencing reveals vegfr as a potential target for car-t cell therapy in chordoma. *Br. J. Cancer* **130**:1609-1620. DOI:10.1038/s41416-024-02635-5

100. Wang K., Osei-Hwedieh D. O., Walhart T. A., et al. (2025). B7-h3 car-t cell therapy combined with irradiation is effective in targeting bulk and radiation-resistant chordoma cancer cells. *J. Immunother. Cancer* **13**. DOI:10.1136/jitc-2024-009544

101. Wu H., Xu Z., Qi M., et al. (2024). Interleukin-7 expression by car-t cells improves car-t cell survival and efficacy in chordoma. *Cancer Immunol. Immunother.* **73**:188. DOI:10.1007/s00262-024-03756-9

102. Bai J., Shi J., Zhang Y., et al. (2023). Gene expression profiling identifies two chordoma subtypes associated with distinct molecular mechanisms and

- clinical outcomes. *Clin. Cancer Res.* **29**:261-270. DOI:10.1158/1078-0432.Ccr-22-1865
103. Yin H., Hu J., Gao J., et al. (2024). Clinical-proteomic classification and precision treatment strategy of chordoma. *Cell Rep. Med.* **5**:101757. DOI:10.1016/j.xcrm.2024.101757
104. Gao J., Hu J. and Meng T. (2025). Protocol for constructing in vivo and in vitro models integrating clinical and proteomic subtypes of chordoma. *STAR Protoc.* **6**:103719. DOI:10.1016/j.xpro.2025.103719
105. AlHammadi S. A., Nagshabandi L. N., Muhammad H., et al. (2025). Mass spectrometry-based proteomics of ffpe tissues: Progress, limitations, and clinical translation barriers. *Clin. Proteomics* **22**:45. DOI:10.1186/s12014-025-09567-z
106. Mischak H., Ioannidis J. P., Argiles A., et al. (2012). Implementation of proteomic biomarkers: Making it work. *Eur. J. Clin. Invest.* **42**:1027-1036. DOI:10.1111/j.1365-2362.2012.02674.x
107. Qi M., Cattaneo G., Camillo C., et al. (2025). Pitfalls and strategies of car-t therapy in solid tumors and implications for chordoma treatment. *Immunotherapy* **17**:735-747. DOI:10.1080/1750743x.2025.2536458
108. Bell A. H., DeMonte F., Raza S. M., et al. (2018). Transcriptome comparison identifies potential biomarkers of spine and skull base chordomas. *Virchows Arch.* **472**:489-497. DOI:10.1007/s00428-017-2224-x
109. Huo X., Ma S., Wang C., et al. (2023). Unravelling the role of immune cells

and fn1 in the recurrence and therapeutic process of skull base chordoma.

Clin. Transl. Med. **13**:e1429. DOI:10.1002/ctm2.1429

110. Ren C., Hornicek F. J., Rosenberg A. E., et al. (2026). Role of extracellular matrix in chordoma progression and therapeutic resistance. *Crit. Rev. Oncol. Hematol.* **218**:105073. DOI:10.1016/j.critrevonc.2025.105073

111. Saito T., Mizumoto M., Oshiro Y., et al. (2024). Systematic review and meta-analysis of particle beam therapy versus photon radiotherapy for skull base chordoma: Trp-chordoma 2024. *Cancers (Basel)* **16**. DOI:10.3390/cancers16142569

112. Bellino S. and La Salvia A. (2025). Methodological challenges and clinical perspectives in evaluating new treatments for ultra rare cancers. *Curr. Med. Res. Opin.* **41**:369-373. DOI:10.1080/03007995.2025.2470735

113. Yada E., Wada S., Yoshida S., et al. (2018). Use of patient-derived xenograft mouse models in cancer research and treatment. *Future Sci. OA* **4**:Fso271. DOI:10.4155/fsoa-2017-0136

114. D'Agati G., Cabello E. M., Frontzek K., et al. (2019). Active receptor tyrosine kinases, but not brachyury, are sufficient to trigger chordoma in zebrafish. *Dis. Model. Mech.* **12**. DOI:10.1242/dmm.039545

115. Torsvik A., Stieber D., Enger P., et al. (2014). U-251 revisited: Genetic drift and phenotypic consequences of long-term cultures of glioblastoma cells. *Cancer Med.* **3**:812-824. DOI:10.1002/cam4.219

116. Huang T., Xu H., Wang H., et al. (2023). Artificial intelligence for medicine:

Progress, challenges, and perspectives. *Innov. Med.* 1. DOI:10.59717/j.xinn-med.2023.100030

FUNDING AND ACKNOWLEDGMENTS

This work was supported in part by the National Natural Science Foundation of China (82522063, 82472786, 82173168). Schematic models were created using Biorender (<http://biorender.com/>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

DWS and TM conceived the review; CCL and TM wrote the manuscript; all authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study.

Table 1 Summary of chordoma treatment strategies: development status and evidence level.

Treatment category	Specific strategy	Development stage	Outcomes	Main limitations	Reference
Local therapy	Carbon ion / proton radiotherapy	Clinical use (specialized centers)	5-year DFS 50% (N=188); 3-year PFS 81% (N=20)	High cost; limited availability; long-term toxicity unknown	81
Targeted therapy	Direct brachyury-targeted agents	Preclinical	T-DARPs, small-molecule binders identified	No clinical-grade compounds yet	52
	Apatinib (VEGFR2 inhibitor)	Phase II	Median PFS 18 months; ORR 25.9% (Choi)	Phase III data lacking; hypertension/proteinuria	90
	Lapatinib (EGFR/HER2 inhibitor)	Phase II completed	Median PFS 6 months; CBR 22%	Modest activity; no RECIST responses	92
	Imatinib (multi-kinase inhibitor)	Off-label / Phase II	minimal PR	Low evidence level; off-label	93
	Palbociclib (CDK4/6 inhibitor)	Phase II completed	median PFS 5.6 months, median OS 24.6 months	Modest single-agent activity	94
	Vismodegib (SMO inhibitor)	Preclinical	G1/S arrest in vitro; xenograft growth inhibition	No clinical trial initiated	96
Immunotherapy	Brachyury vaccine	Phase I/II completed	Immunogenic platform; 1 PR (IV, Ph I) and 2	Limited single-agent cytotoxicity (ORR <8%)	51,97

					PRs (with RT, Ph				
					II, mRECIST).				
		Pembrolizumab	Case report		One durable PR		Lacks prospective data	98	
		(anti-PD-1)							
		CAR-T (B7-H3,	Preclinical		Effective	in	No clinical trial initiated	99,100	
		VEGFR)			xenografts;				
					synergy	with			
					radiation				
		YL-13027 (p-	Phase I		SD in 3	SBC	Small sample size	65	
		EMT inhibitor,	completed		patients, no severe				
		targets TGF- β)			AEs				
		Denosumab	Preclinical		Suppresses		Requires prospective	103	
		(BM-dominant)			osteoclastogenesis		validation		
		S-Gboxin	Preclinical		Targets		Only validated in	103	
		(OXPHOS-high)			mitochondrial		preclinical models		
					metabolism				
		Anlotinib	Preclinical		RTK inhibition		subtype-specific activity	103	
		(MET-mediated)					unproven		

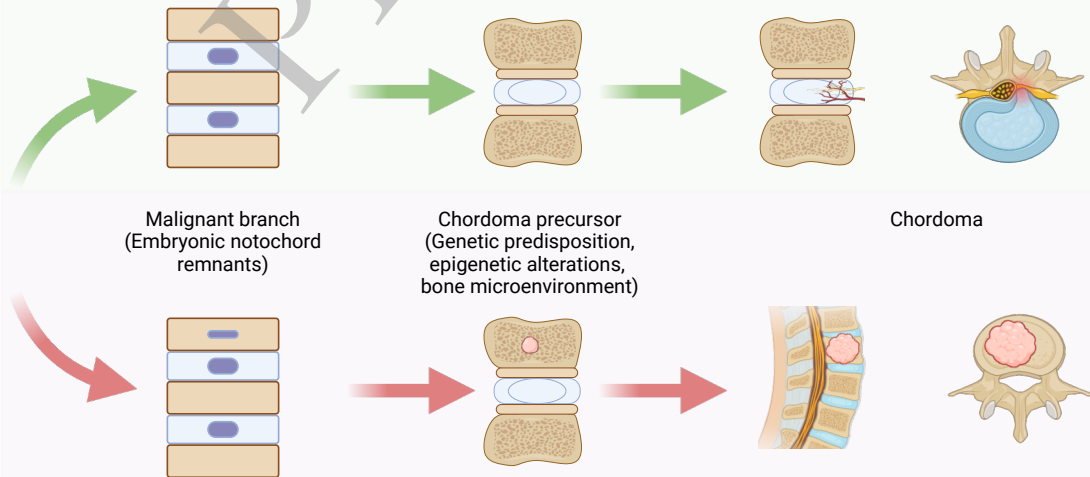
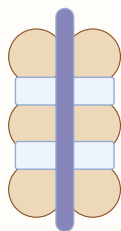
Abbreviations: CBR, clinical benefit rate; ORR, objective response rate; PFS, progression-free survival; PR, partial response; OS, overall survival; RECIST, Response Evaluation Criteria in Solid Tumors; IV, intravenous; RT, radiotherapy; SD, stable disease; AE, adverse event(s).

Table 2. Branching of notochord fate: comparison between NP formation and chordoma development.

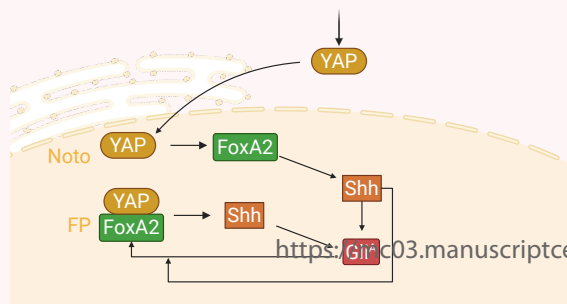
This table compares notochord cell fate in normal differentiation (NP formation) versus malignant transformation (chordoma development) across five dimensions: definition, cellular origin, stage of occurrence, key drivers, and microenvironmental features. It illustrates the molecular basis and translational value of distinct fates under the "shared notochord lineage" perspective.

Feature	NP formation (normal branch)	Chordoma development (malignant branch)
Definition	Notochord cells persist and differentiate in the IVD region, forming the NP and providing cushioning function	Notochord cells remain in the vertebral body and undergo malignant transformation to form chordoma
Cellular origin	Notochord cells are compressed into the intervertebral space	Embryonic notochord remnants
Stage of occurrence	Late embryonic development	Peak incidence at 50 years of age; rarely affects patients under 40, especially children
Key drivers	Mechanical compression, hypoxic microenvironment, TGF- β signaling	Genetic predisposition, epigenetic alterations, bone microenvironment
Microenvironmental features	Avascular, hypoxia, high hydrostatic pressure	Bone microenvironment-dominated; accompanied by angiogenesis and nerve invasion in advanced stages; rich in immune cell infiltration

Notochord



Mechanical signals



Autophagy ↓

ATG5

ATG5 mRNA

SMARCB1

ATG5 Promoter

Proteomics-based
subtyping of chordoma

BM-dominant

OXPHOS-high

MET-mediated

Denosumab

S-Gboxin

Anlotinib

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28



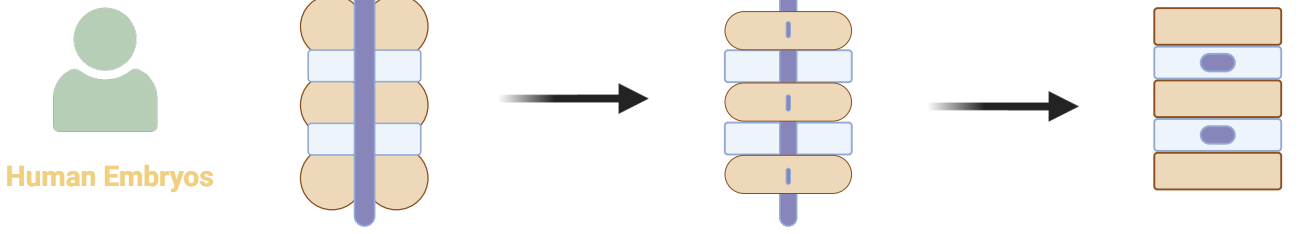
Human Embryos

Day 19

Day 23

Day 25

B



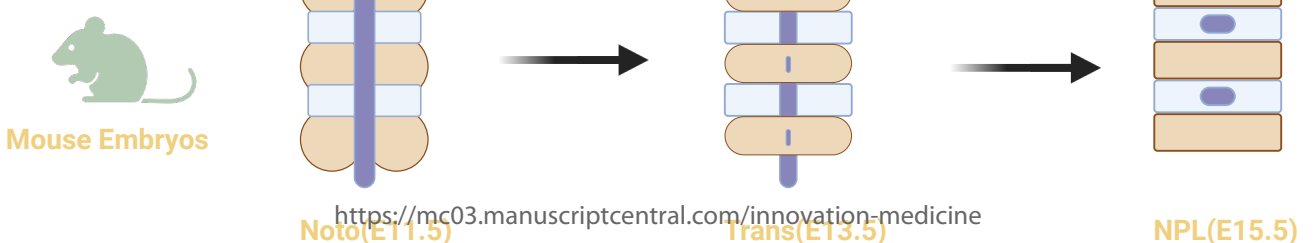
Human Embryos

Noto
(7th week)

Trans
(8-9th weeks)

NPL
(10-11th weeks)

C

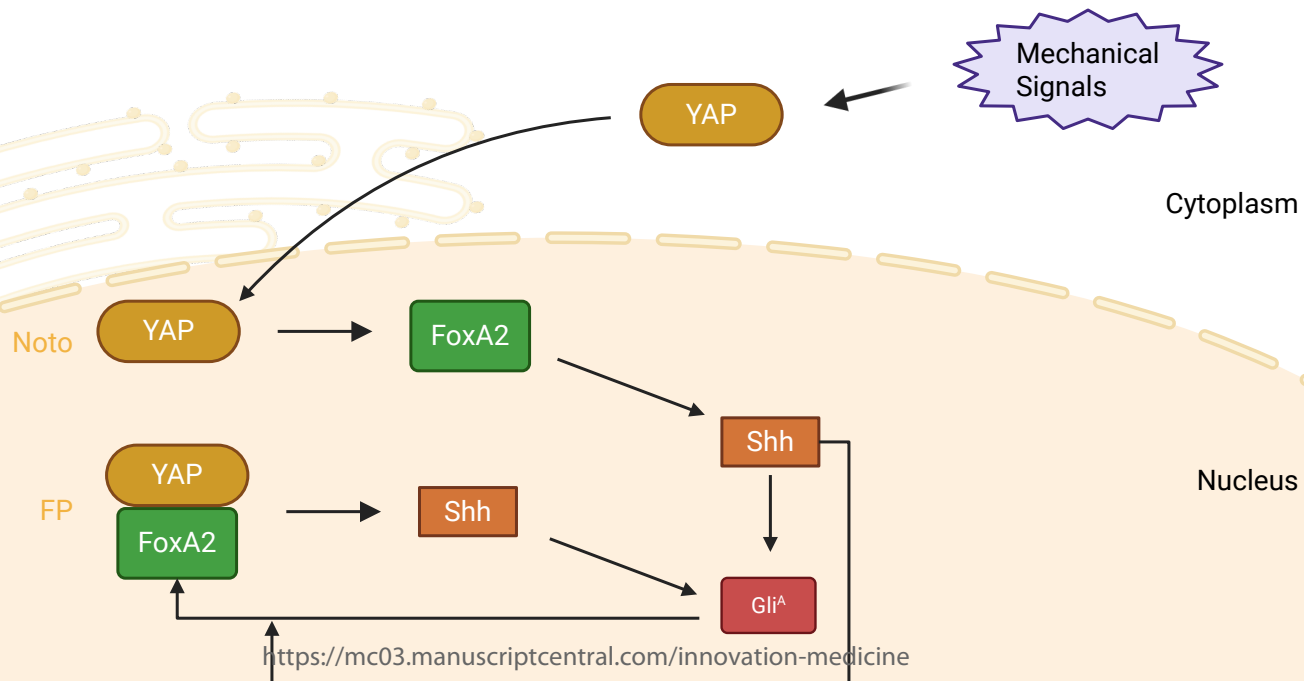


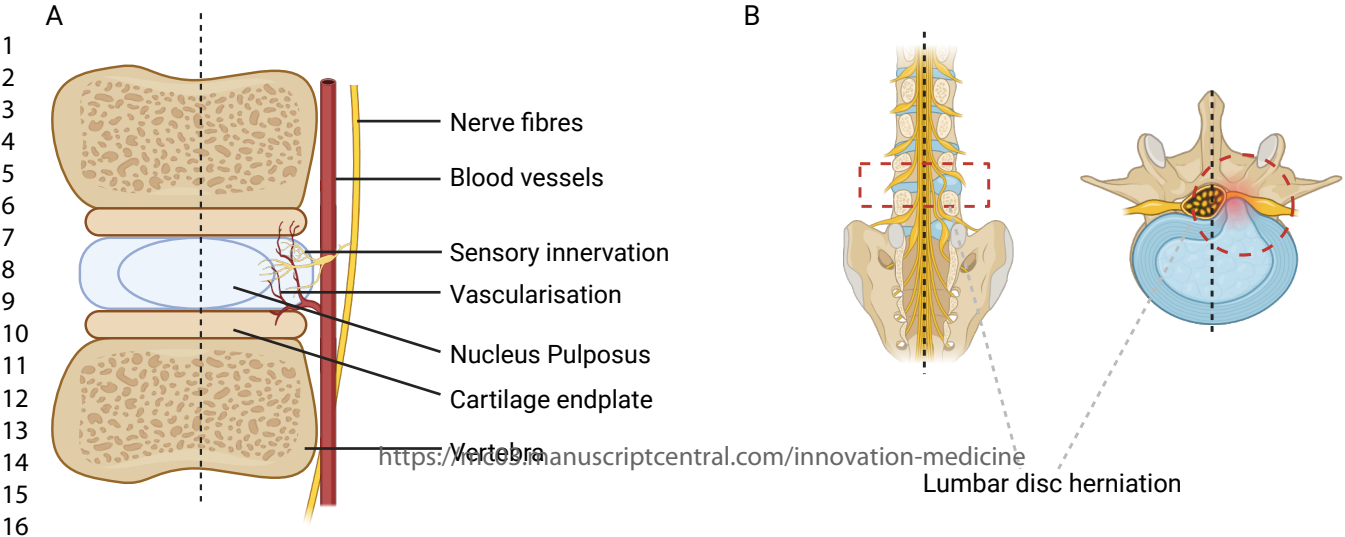
Mouse Embryos

Noto(E11.5)

Trans(E13.5)

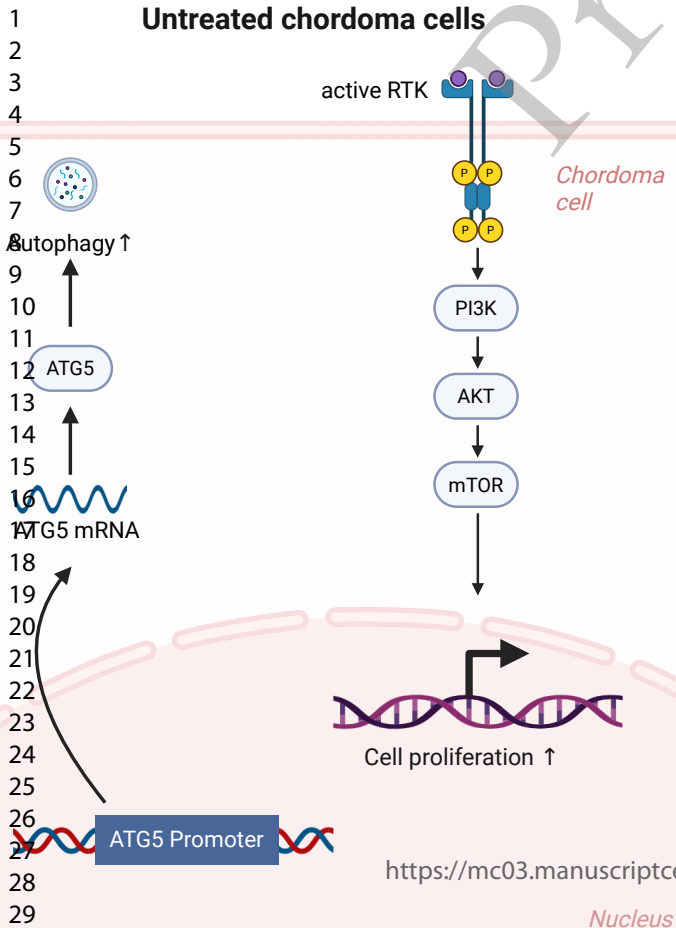
NPL(E15.5)





Some Therapeutic Targets for Chordomas

Untreated chordoma cells



Treated chordoma cells

