Journal of Translational Medicine

RESEARCH

Open Access

Alternative lengthening of telomeres confers favorable prognosis in chondrosarcomas

Ji-Yong Sung[1*], Jin-Hong Kim[1*] and Yi-Jun Kim[2,3*]

Abstract

Background Cancer cells achieve replicative immortality through telomere maintenance mechanisms (TMMs), primarily via telomerase activation or alternative lengthening of telomeres (ALT). Sarcomas frequently employ the ALT pathway, which traditionally correlates with adverse clinical outcomes. However, chondrosarcomas represent a unique context where the role and prognostic significance of ALT remain largely unexplored.

Methods We performed comprehensive analyses of single-cell RNA-sequencing data from patients with chondrosarcoma and integrated this with 90 bulk RNA-seq datasets. This approach enabled detailed characterization of TMM at single-cell resolution, identification of ALT-specific signatures, and evaluation of the tumor microenvironment in chondrosarcomas.

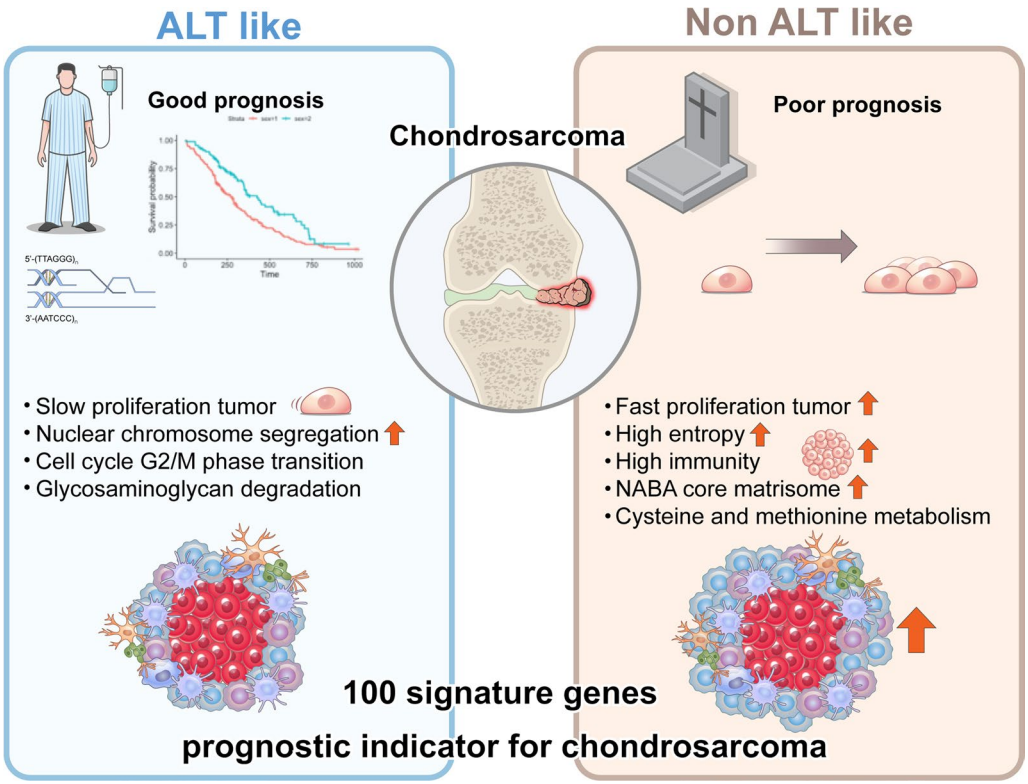
Results Patients with ALT-like chondrosarcomas exhibited significantly improved survival compared to those with non-ALT-like chondrosarcomas. Analysis of the tumor immune microenvironment revealed distinct metabolic and immune landscapes between the ALT-like and non-ALT-like groups. Single-cell analysis showed that high-entropy stem-like cells in high-grade chondrosarcomas predominantly adopted telomerase activation over the ALT pathway as their TMM. Additionally, we identified a 100-gene signature that reliably distinguishes ALT-like chondrosarcomas, providing a robust molecular marker for classification and prognosis.

Conclusions Our study reveals ALT-like state as a marker of favorable prognosis in chondrosarcomas—contrasting with its typically adverse implications in other sarcomas. We establish a robust 100-gene signature that reliably identifies ALT-like chondrosarcomas and characterize their distinct immune microenvironment profile.

Keywords Alternative lengthening of telomeres, Prognosis, Chondrosarcoma, Tumor immune microenvironment, Signature genes

*Correspondence:
Ji-Yong Sung
5rangepineapple@gmail.com
Jin-Hong Kim
jinhkim@snu.ac.kr
Yi-Jun Kim
yijunkim@ewha.ac.kr
Full list of author information is available at the end of the article

Graphical abstract



Background

Telomeres, defined as the repeated DNA–protein complexes (TTAGGG) at the chromosomal termini, are essential for cancer cell viability. In most cancers, these complexes are sustained by an enzyme called telomerase [1]. Alternative lengthening of telomere (ALT) is a recombination-based telomere maintenance mechanism (TMM) used by 10–15% of human malignancies. ALT is prevalent in malignancies of mesenchymal origin and frequently indicates a poor prognosis [2]. In the absence of telomerase, ALT is induced by spontaneous double-stranded DNA damage, persistent replication stress, and telomere attrition [3]. Unresolved replication stress and telomere degradation cause double-stranded DNA damage [4]. Chondrosarcomas are a significant category of sarcomas, ranking second in prevalence to osteosarcomas. The term 'chondrosarcoma' refers to a heterogeneous collection of malignant tumors originating from chondroid cells in the appendicular and axial skeletons. It constitutes approximately 11% of primary malignant bone neoplasms [5, 6]. Sarcoma, which is recognized as a representative ALT-positive cancer, shows poor clinical outcomes; however, glioblastoma multiforme (GBM) and liver hepatocellular carcinoma (LIHC), which have high ALT levels, exhibit a favorable prognosis [7]. ALT activation in cancer cells triggers substantial telomere elongation, resulting in characteristically long telomeres [8]. The development of ALT is associated with poor prognosis, resulting in a refractory cancer form [9, 10]. Antitelomerase therapy occasionally induces ALT and mitochondrial adaptive mechanisms in cancer cells [11]. ALT-positive cancer cells become refractory because of enhanced mitochondrial bioenergetics and the inhibition of cell death pathways [12]. Most ALT studies have focused on identifying therapeutic targets that inhibit ALT and reduce the number of ALT-positive cancer cells in response to drug therapy. ALT renders cancer cells hypersensitive to ataxia telangiectasia and Rad3-related inhibitors [13]. In contrast, research on ALT-positive cancers with improved prognosis is lacking, and the processes underlying the favorable prognosis in GBM or LIHC with high ALT levels remain unelucidated. In GBM, patients with elevated ALT levels have favorable outcomes, and ALT serves as a prognostic indicator [14]. In this study, we aimed to evaluate the prognostic value

of ALT and assess the tumor immune microenvironment in chondrosarcoma. To our knowledge, this is the first study to classify patients with chondrosarcomas into ALT and non-ALT groups and revealed that the ALT group had better outcomes. The distinctive features of ALT in chondrosarcomas were identified via tumor microenvironment and metabolic reprogramming assessments. Our novel findings will enhance the understanding of precision medicine therapies for chondrosarcoma.

Methods

Single-cell RNA-sequencing analysis

Cell-type classification of the chondrosarcoma single-cell data was performed using the Seurat R package (version 4.4.1) [15] to distinguish cell types using the default parameters. Cell-type marker genes were categorized according to the cell type in the literature [16]. The StemID tool [17] was used to identify stem-like cells in single cells, and a p-value < 0.05 was calculated as the standard. CellChat was used to analyze cell–cell interactions [18].

Bulk RNA-seq analysis

For the survival analysis of signature genes, Gene Expression Profiling Interactive Analysis 2 [19] was used for The Cancer Genome Atlas (TCGA) data, and R package "survival" was used for chondrosarcoma bulk RNA-seq. Metascape [20] was used for Gene Ontology and protein–protein interaction (PPI) analyses; for the gene enrichment test, the Gene Set Variation Analysis algorithm [21] was used and run over 1,000,000 times for statistical accuracy. A gene enrichment test was used for the signature score and Kyoto Encyclopedia of Gene and Genome (KEGG) metabolic pathway. To estimate immune cell composition from bulk RNA-seq data, we applied three widely used computational deconvolution methods: CIBERSORTx, EPIC, and xCell. All analyses were performed using default parameters provided by each tool, and normalized gene expression matrices were used as input. These complementary approaches enabled a robust characterization of the tumor immune microenvironment.

Results

High expression of high-entropy stem-like signature genes predicts poor prognosis in chondrosarcoma

We analyzed TMM and the tumor immune microenvironment using single-cell RNA-seq (GSE184118) and bulk mRNA profiling by an array of cartilage tumors (https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-7264) of 90 patients with chondrosarcomas (Fig. 1a). We classified single-cell data into high-grade and benign/low-grade. Using StemID [17], we calculated the entropy of single cells from patients with high-grade tumors to identify stem-like cells. Cell type distribution was analyzed across clusters with varying entropy levels. High-grade tumor cells were classified into two types (H1 and H2). In high-grade tumors, Clusters 13, 19, 25, 27, 1, and 2 (entropy high order) predominantly contained activated stem cells and H2 cancer cells with high entropy. While these clusters showed diverse cell type composition, they were characterized by high-entropy cancer cells. Within the same high-grade category, H1 cancer cells in Clusters 4, 5, 6, 7, and 26 exhibited relatively lower entropy than did H2 cancer cells. When benign and low-grade data were combined and examined, the combined sample had fewer high-entropy activated cells than did the high-grade samples. Clusters 4, 9, 20, 12, and 15 corresponded to high-entropy stem-like cells, and Cluster 19 belonged to the low-entropy group. Different cell type distributions were verified based on the cluster. These findings revealed the substantially expressed genes with differential expression in high-entropy stem-like cells (Fig. 1b). We selected 61 genes from the benign/low-grade group and 146 from the high-grade group. Survival analysis was performed on the TCGA sarcoma data using stem-like signature genes based on each tumor grade. In the sarcoma data with seven molecular subtypes (undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, uterine leiomyosarcoma, soft tissue leiomyosarcoma, myxofibrosarcoma, synovial sarcoma, and malignant peripheral nerve sheath tumor), high expression of signature genes was associated with good prognosis (Fig. 1c). Conversely, a similar analysis in the chondrosarcoma cohort showed that the group

(See figure on next page.)

Fig. 1 High expression of high entropy stem-like signature genes predicts poor prognosis in chondrosarcoma. **a** The analysis pipeline for this study. **b** T-distributed stochastic neighbor embedding plot for fibroblasts and cancer stem cells in high grade (left), bar graph of entropy (middle), and bar graph of cell type in high grade and benign/low grade (right). **c** Kaplan–Meier plots showing the overall survival rates of the high- and low-SIG87 groups of TCGA sarcoma (left) and the SIG61 groups of TCGA sarcoma (right). **d** Kaplan–Meier plots showing the overall survival rates for the high- and low-SIG146 groups in chondrosarcoma (left) and the SIG61 groups in chondrosarcoma (right). ALT, Alternative Lengthening of Telomeres; SIG61, Stem-like Signature Genes 61; SIG87, Stem-like Signature Genes 87; SIG146: Stem-like Signature Genes 146; TCGA, The Cancer Genome Atlas; t-SNE: T-distributed Stochastic Neighbor Embedding

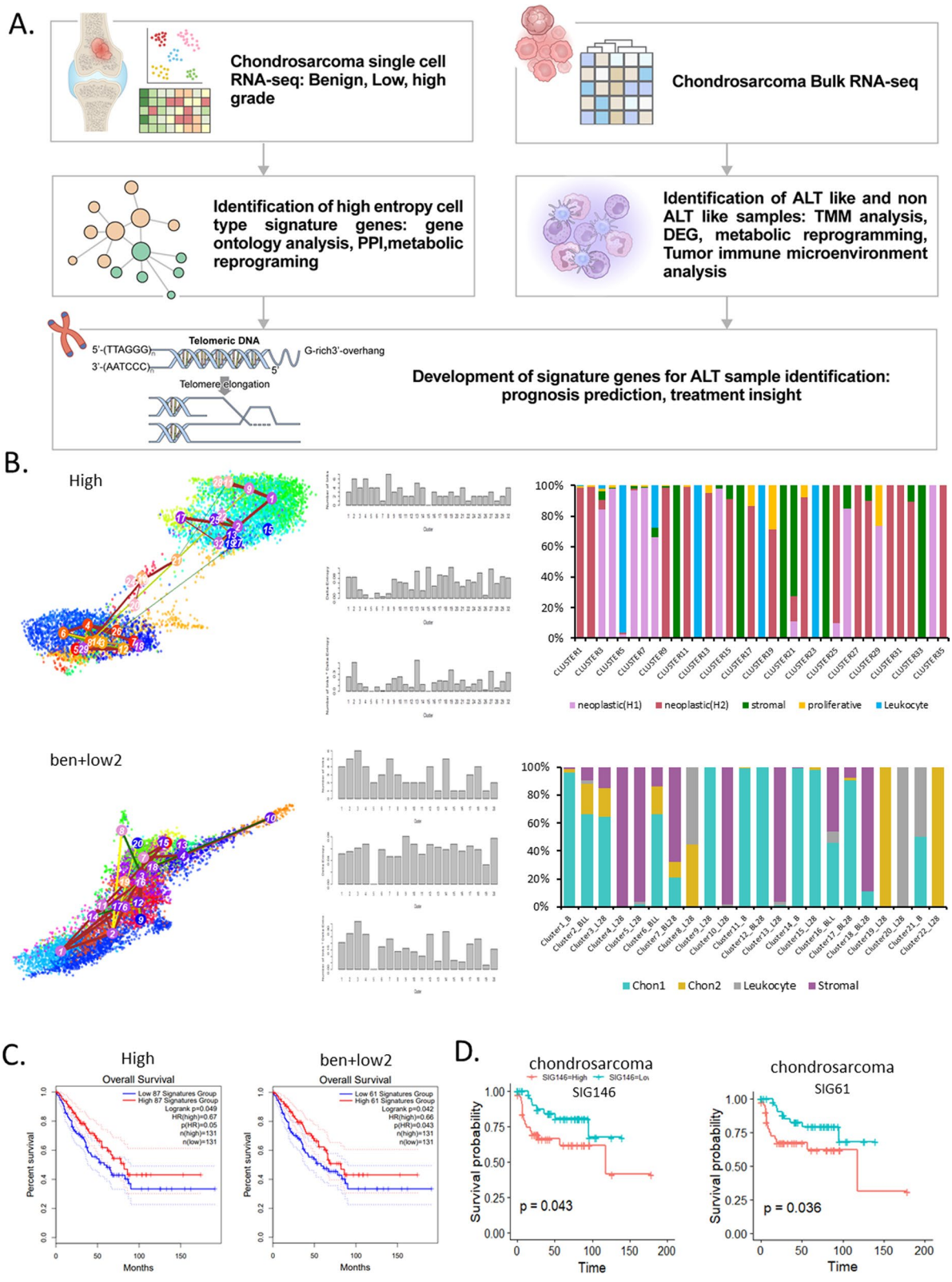


Fig. 1 (See legend on previous page.)

exhibiting high signature gene expression had a markedly poor prognosis (SIG146: $p=0.043$, SIG61: $p=0.036$) (Fig. 1d).

Entropy, stemness, and TMM vary according to the cell type at the single-cell level

We analyzed the TMM using single-cell data. We performed a TMM analysis based on the pathway gene list [7], which showed different TMM patterns depending on the high-entropy stem-like cluster (Fig. 2a). In clusters with low telomerase activity, the activity of the ALT instability pathway was elevated. Different TMM activities could be validated based on the tumor grade and cell type. In high-grade malignancies, H1 and H2 cells exhibited distinct ALT pathway activities. The activities of the ALT instability and ALT homologous recombination pathways were elevated in H2 and H1 cells, respectively. TMM exhibited the traits of various cancer cells within the same grade (Fig. 2b, c). Our study indicated that H2 cells exhibited significantly high entropy, indicating an elevated stemness level. Cancer cells exhibiting distinct features within the same grade demonstrated varying ALT pathway activities. Furthermore, we confirmed elevated telomerase activity in Chon2 cancer cells within the benign and low-grade categories. ALT activity was elevated in stromal cells and leukocytes, with pronounced chromatin decomposition and heightened activity in the ALT instability pathway. Gene Ontology analysis revealed that the highly expressed genes in the stem-like cells within the high-grade group are predominantly associated with rheumatoid arthritis, cell activation regulation, bacterial response, and positive cytokine production regulation. The genes in the benign/low-grade group are enriched in inflammatory responses, rheumatoid arthritis, and processing and presentation of exogenous antigens, among others (Fig. 2d, e). We validated the PPI of this gene signature, which was associated with immunity (Fig. 2f, g). We examined the metabolic energy utilized by the high-entropy stem-like cells in the high-grade clusters. Metabolic heterogeneity varies according to the cellular state. Clusters 13, 19, 27, 17, and 25 exhibited high entropy, whereas Cluster 11 showed relatively low entropy. However, within the lineage tree, high-entropy cells may acquire low-entropy states. Upon completion of Cluster 11, it exhibited elevated activity across most metabolic pathways (Fig. 2h). The results validated the characteristics of the TME through the existence of varying TMM heterogeneity and metabolic heterogeneity [22] based on the cell type and state.

Differences between tumor microenvironments in ALT-like and non-ALT-like chondrosarcomas

We used bulk RNA-seq to categorize samples into four groups based on the TMM pathway activity identified by Sung et al. [7] and conducted a survival analysis. The undefined TMM group exhibited a favorable prognosis similar to that of the ALT-like group, whereas the prognosis of the group with only telomerase activity was poorer than that of the group with concurrent telomerase and ALT activities ($p=0.00015$) (Fig. 3a). Additionally, we classified the samples into two types, ALT-like and non-ALT-like ($p=0.0045$) (Fig. 3b). The chondrosarcoma ALT-like group showed decreased telomerase pathway activity relative to that of the non-ALT-like group (tel_tert: $p=6.7e\text{-}07$, tel_terc_dkc1: $p=0.017$) (Fig. 3c). In the ALT-like group, the activities of ALT chromatic decomposition and the ALT instability pathway were significantly elevated (ALT_chr: $p=0.0032$, ALT_ins: $p=0.0011$) (Fig. 3c). In the ALT-like group, the highly expressed genes were predominantly enriched in nuclear chromosomal segregation, control of chromosome segregation, assembly of non-membrane-bounded organelles, and meiotic cell cycle processes (Fig. 3d). The non-ALT-like group showed enrichment in pathways, such as skeletal system development, NABA core matrisome, mesodermal cell differentiation, and negative regulation of growth (Fig. 3d). The transcription factors E2F3, E2F1, and TP53 were predominantly identified in the ALT-like group (Fig. 3e). Furthermore, PPI analysis revealed an association with immunity in the ALT-like group (Fig. 3f). We conducted a deconvolution analysis to examine the tumor immune milieu. B-cell plasma cells, CD8 T cells, monocytes, activated myeloid dendritic cells, memory CD4 T cells, common lymphoid progenitors, Th1 CD4 T cells, and resting natural killer (NK) cells exhibited markedly elevated activity under non-ALT-like

(See figure on next page.)

Fig. 2 Entropy, stemness, and TMM vary according to the cell type at the single-cell level. **a** Box plot of the telomerase TERT pathway activity in high-grade entropy clusters (left) and box plot of the ALT instability pathway activity in high-grade clusters (right). **b** Box plot of the telomere maintenance mechanism pathway activity in high-grade clusters. **c** Boxplot of the telomere maintenance mechanism pathway activity in benign/low-grade clusters. **d** Gene Ontology network in enriched genes of high-grade clusters. **e** Gene Ontology network in enriched genes of benign/low-grade clusters. **f** Protein–protein interaction (PPI) of the enriched genes in high-grade clusters. **g** PPIs of the enriched genes in the benign/low-grade clusters. **h** Metabolic pathway activity in high-grade entropy clusters. ALT: Alternative Lengthening of Telomeres; TERT: Telomerase Reverse Transcriptase; TMM: Telomere Maintenance Mechanism

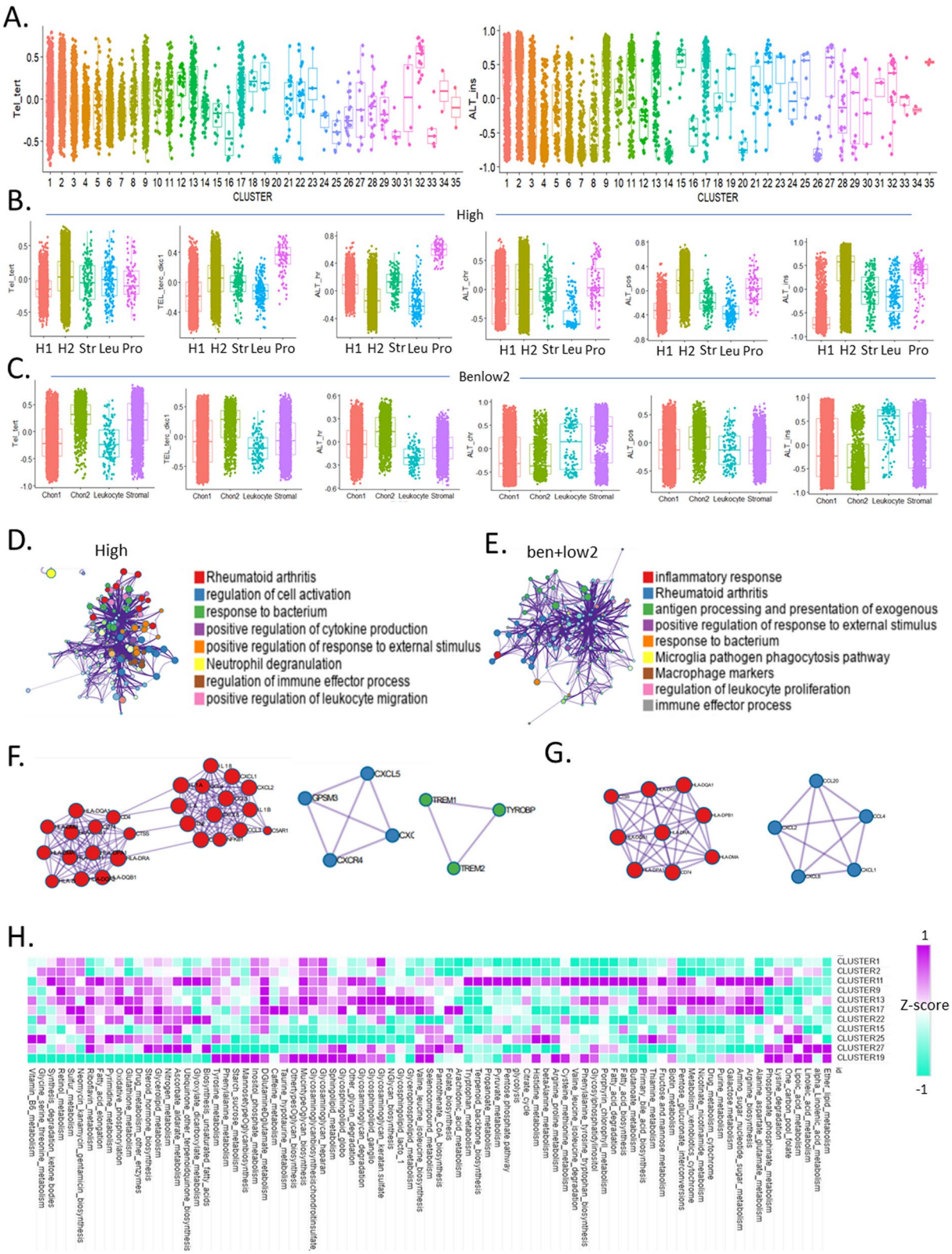


Fig. 2 (See legend on previous page.)

conditions (Fig. 3g). Conversely, in the ALT-like group, macrophages ($p=0.036$), B cells ($p=0.0068$), M2 macrophages ($p=0.024$), cancer-associated fibroblasts ($p=0.016$), stroma scores ($p=0.0054$), and endothelial cells ($p=0.027$) were significantly elevated. We examined the functionality of the KEGG84 metabolic pathway. In the ALT-like group, the activities of D-glutamine and D-glutamate metabolisms, glycosaminoglycan degradation, glycosaminoglycan heparin, glycosaminoglycan biosynthesis, chondroitin sulfate, and sulfur metabolism pathways were elevated. In contrast, in the non-ALT group, the metabolism of fructose, mannose, galactose, cysteine, methionine, ubiquinone, and other terpenoid quinones and the activities of neomycin, kanamycin, and gentamicin biosynthetic pathways were elevated (Fig. 3h).

In the ALT-like group, galactose metabolism was strongly positively correlated with naïve B cells, CD8 T cells, monocytes, resting NK cells, and common lymphoid progenitors. CD4 memory T cells, common lymphoid progenitors, CD4 Th1 T cells, and CD4 Th2 T cells exhibited strong positive correlations with the glycosaminoglycan heparin (Fig. 3i). Metabolic heterogeneity was positively correlated with various immune cells. In the non-ALT cell-like group, common lymphoid progenitor cells showed the strongest positive correlation with diverse metabolic activities (Fig. 3j). CD8 T cells were positively correlated with D-glutamine, D-glutamate, fructose, and mannose metabolisms. Endothelial cells were the primary metabolic pathways with the highest activity in the ALT-like group. Metabolic pathways that showed high activity in the ALT-like and non-ALT-like groups were positively correlated with the immune cells of each group. These characteristics confirmed that immune cells used metabolic energy differently in the ALT- and non-ALT-like groups.

SIG100 score is an indicator of ALT levels in chondrosarcoma and shows a poor prognosis in the high-expression group

We identified 100 markedly distinct genes using bulk RNA-seq (ALT-like vs. non ALT-like) differential expression analysis. We assessed SIG100 scores based on the clustering derived from the entropy of single-cell data and found that clusters with lower entropy (Clusters 4, 5, and 7) had higher SIG100 scores. This is possibly non-ALT, similar to H1 cancer cells, which have low entropy despite being the same high-grade cancer cell type. H1 cancer cells are predominantly non-ALT-like and have a poorer prognosis than do H2 cancer cells. In the benign/low-grade group, the SIG100 score was predominantly increased in the high-entropy cluster, indicating a non-ALT-like profile and poor prognosis. Consequently, for high-grade high-entropy clusters, telomerase and ALT activities were increased, categorizing these cases as non-ALT-like, which may indicate a particularly poor prognosis. In contrast, clusters with high stemness and low SIG100 scores had a high possibility of being ALT-like cells, suggesting a good prognosis. SIG100 expression was increased in cells exhibiting high entropy in low/benign-grade cancers, as well as in low-entropy cells with low stem-like features in high-grade cancers. A high score in the high-grade cluster does not indicate a favorable prognosis; however, cells exhibiting elevated stemness in high-grade cancers were associated with a good prognosis (Fig. 4a). These prediction scores depend on the stemness and the cell type. Highly proliferative cells exhibited the highest SIG100 scores, followed by neoplastic H1 cells. The two cell types were identified to participate in Pleiotrophin-NLC-ligand-receptor signaling (Fig. 4b). In contrast, benign/low-grade tumors had the highest SIG100 expression in leukocytes and non-ALT-like cells. Leukocytes are linked to MIF-CD74 + CD44 ligand-receptor signaling (Fig. 4c). The features of non-ALT-like cells in chondrosarcomas vary according to the tumor grade. Through the analysis of bulk samples, we established that SIG100 exhibited significantly elevated expression in the non-ALT group (Fig. 4d). In the chondrosarcoma dataset, high SIG100 expression was correlated with a poor prognosis ($p=0.0015$) (Fig. 4e). In the TCGA sarcoma dataset, characterized by diverse molecular subgroups, increased expression was correlated with unfavorable outcomes (Fig. 4f). These results provide insights into developing personalized therapeutic strategies.

(See figure on next page.)

Fig. 3 Differences between ALT-like and non-ALT-like tumor microenvironments in chondrosarcoma. **a** Kaplan–Meier plots showing the overall survival rates of the TMM groups (ALT, NDTMM, Telomerase + ALT, telomerase) in chondrosarcoma. **b** Kaplan–Meier plots showing the overall survival rates of the ALT-like and non-ALT-like groups in chondrosarcoma. **c** Box plot of TMM activity in the ALT-like and non-ALT-like groups. **d** The network of enriched genes in the ALT-like (left) and non-ALT-like (right) groups. **e** Bar graph of the transcription factors in the ALT group. **f** PPIs in the ALT-like group. **g** Box plot for immune cell activity in the ALT-like and non-ALT-like groups. **h** Enriched metabolic pathway activities in the ALT-like and non-ALT-like groups. **i** Correlation between immune cells and metabolic pathways in the ALT-like group **j** Correlation between immune cells and metabolic pathways in the non-ALT-like group. ALT: Alternative Lengthening of Telomeres; NDTMM: Non-Detectable Telomere Maintenance Mechanism; TMM: Telomere Maintenance Mechanism

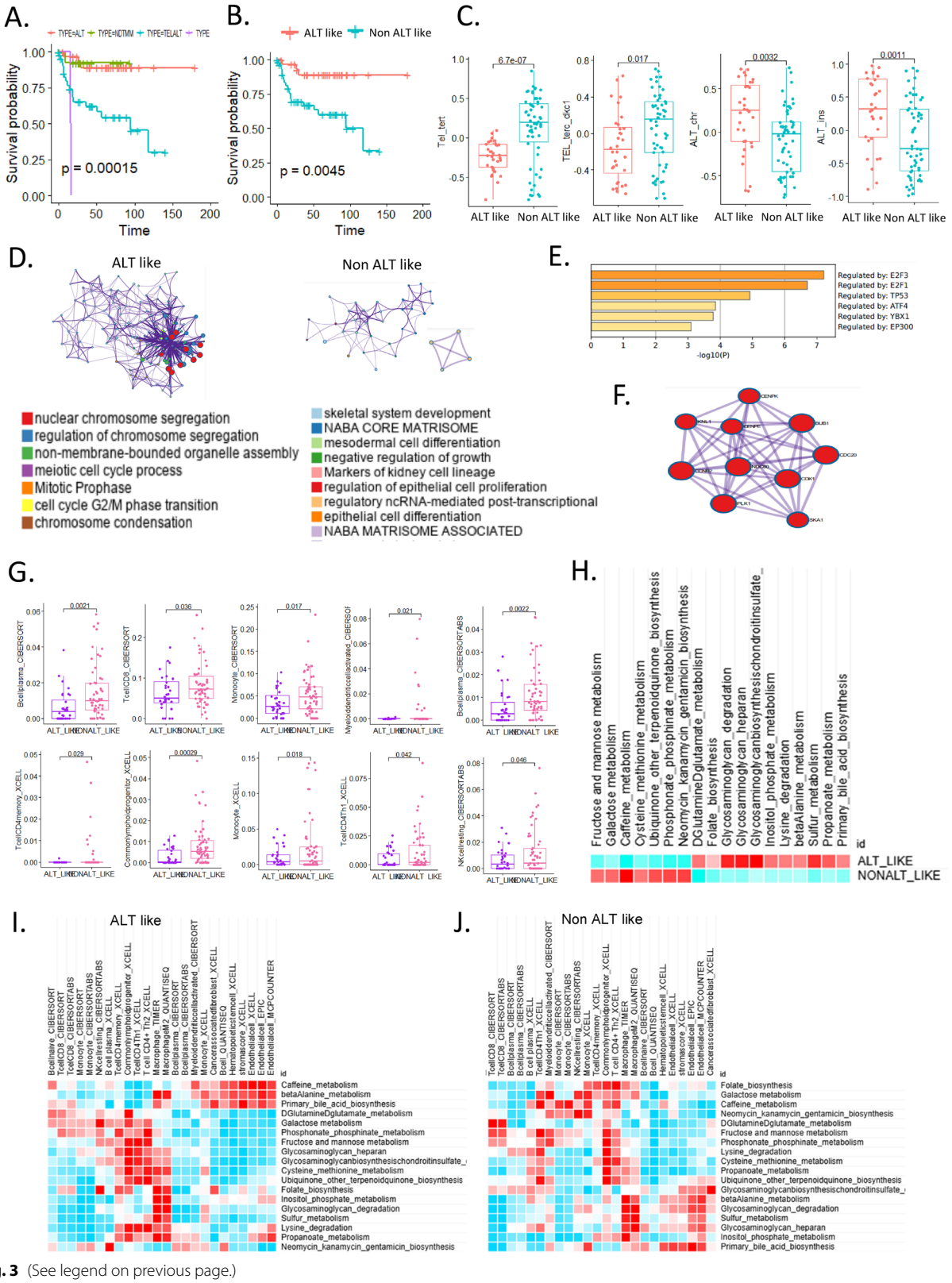


Fig. 3 (See legend on previous page.)

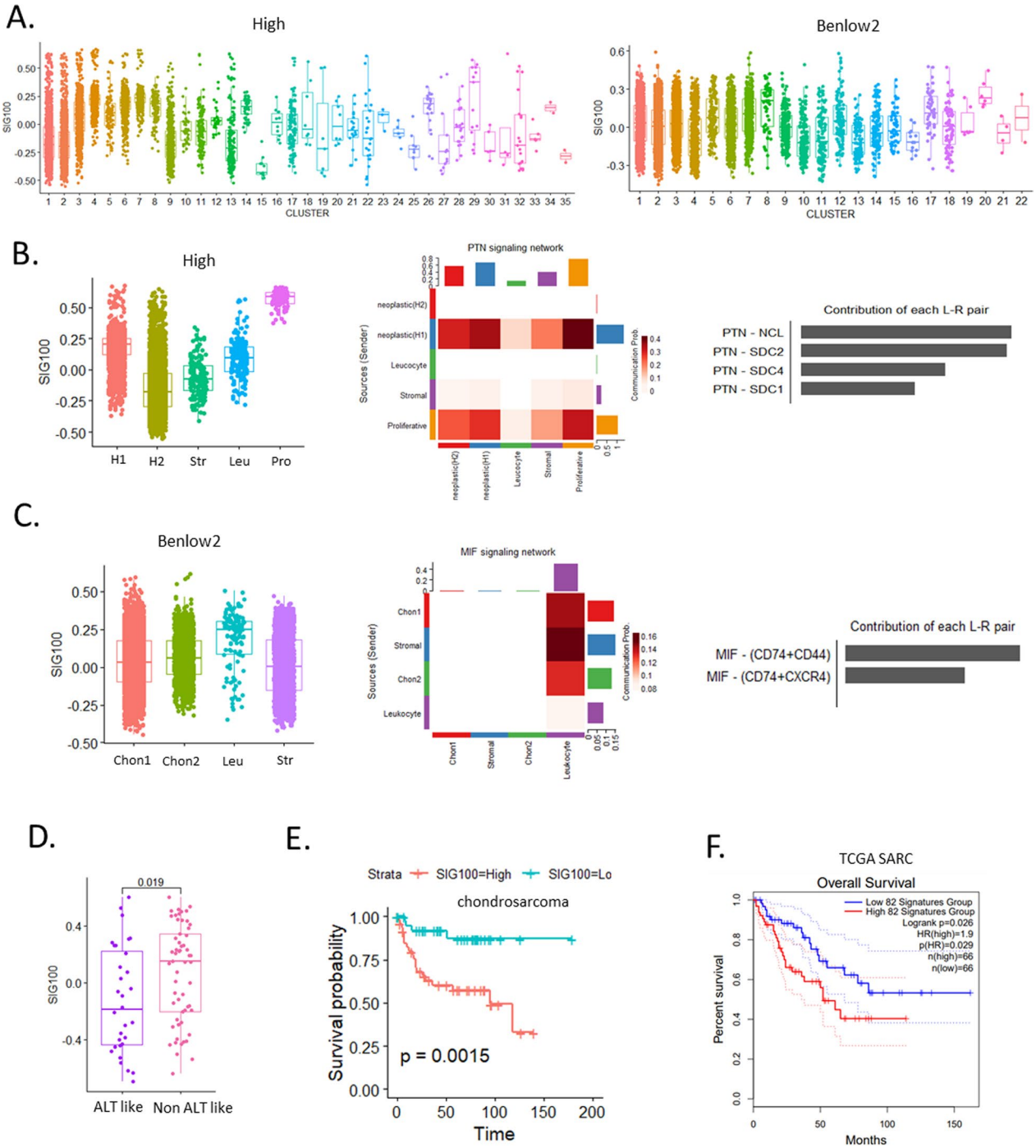


Fig. 4 SIG100 is an ALT indicator for chondrosarcoma and shows a poor prognosis in the high-expression group. **a** Box plot of SIG100 in the high-grade (left) and benign/low-grade (right) groups. **b** Box plot of SIG100 in the cell types of the high-grade group (left). PTN signaling network of the non-ALT-like group in the high-grade group (middle); bar graph of the ligand-receptor pairs in the high-grade group. **c** Box plot of SIG100 in the cell types of the benign/low-grade group (left). MIF signaling network of the non-ALT-like group in the high-grade group (middle); bar graph of the ligand-receptor pairs in the benign/low-grade group. **d** Box plot of SIG100 in the ALT-like and non-ALT-like groups. **e** Kaplan–Meier plots showing the overall survival rates for 82 SIG (SIG100) in the chondrosarcoma cohort. **f** Kaplan–Meier plots showing the overall survival rates for 82 SIG (SIG100) in the TCGA sarcoma data. ALT: Alternative Lengthening of Telomeres; MIF: Macrophage Migration Inhibitory Factor; PTN: Pleiotrophin; SIG: Stem-like Signature Genes; TCGA: The Cancer Genome Atlas

Discussion

Over two decades ago, Hanahan and Weinberg recognized replicative immortality as a hallmark of cancer [23]. TMM has been investigated in several cancer types [24]; however, there are limited studies aimed at identifying marker genes that distinctly differentiate TMM in each specific cancer type. In this study, we aimed to evaluate the prognostic value of ALT and assess the tumor immune microenvironment in chondrosarcoma. We found that ALT is a prognostic indicator for patients with chondrosarcoma. The pan-sarcoma landscape of the telomeric content showed that alterations in *RAD51B* and *GID4* were associated with a high telomeric content [25]. Although there are few TMM studies on chondrosarcoma, chondrosarcoma is not characterized by detectable telomerase activity [26]. In this study, we identified a signature gene that distinguished the ALT status in chondrosarcoma. When ALT-positive cancer develops, it evolves into a recalcitrant tumor characterized by strongly drug-resistant cancer cells. First, ALT-positive cancers exhibit pronounced reproductive potential and persist through the process of immortalization. ALT-positive cancers have a markedly unfavorable prognosis. Conversely, there are conditions where ALT-positive cancers have a favorable prognosis, e.g., GBM. The onset of ALT-positive cancer induces mitochondrial malfunction, resulting in the appropriation of energy that should be utilized by adjacent cells for bioenergetic metabolism. This mechanism makes ALT tumors strong and aggressive. We assessed chondrosarcoma TMM and examined their metabolic and immunological microenvironments by comparing the ALT-like and non-ALT-like tumor groups [27]. However, chondrosarcoma had favorable ALT outcomes.

These characteristics were the same as those that showed a good prognosis in the ALT group in GBM and LIHC in our previous study [7]. The investigation indicated that the most significant mechanism was similar to nuclear segregation, and this characteristic is believed to originate from an organ-specific cellular specialization that cannot reproduce. TMM positively influenced this non-regenerative cell characteristic, confirming that the cell activity was rather low. In other solid tumors, increased stemness was correlated with elevated cell entropy and heightened cellular activity. However, the high-entropy cells did not consistently indicate ALT. ALT-mediated telomere elongation is occasionally observed in a dormant state with minimal activity. Conversely, when telomerase and ALT activities are elevated, cellular activity is considered high. We investigated the cause of elevated immune activity in the ALT-like group at the single-cell level of the chondrosarcoma tumor and its TME. The two groups showed distinct metabolic variability, and their interactions with immune cells were defined. These results can be compared with the energy metabolism in the high-entropy cell group revealed by the single-cell analysis. We acknowledge that the prognostic impact of ALT-like characteristics may vary depending on tumor stage, grade, or the surrounding microenvironment. In our study, we observed variability in SIG100 expression and ALT-like status across different tumor grades, suggesting that the functional consequences of ALT activation may be context-dependent. While this study primarily focused on high-grade tumors due to dataset availability, further investigation using stratified cohorts and integrative analyses could elucidate how tumor-intrinsic and microenvironmental factors modulate the prognostic significance of ALT. Future work incorporating larger, stage-specific datasets or spatial transcriptomics may provide a more comprehensive understanding of these dynamics.

Although mechanistic links between ALT-like TMM and the immune or metabolic landscape in chondrosarcoma remain unclear, previous studies in glioblastoma—a known immune cold tumor—have reported increased immune activity in ALT-positive tumors. While similar evidence is lacking in chondrosarcoma, we hypothesize that ALT activation may influence mitochondrial function or metabolic reprogramming in ways that modulate tumor behavior.

Our study has some limitations. Validation in larger cohorts of patients with chondrosarcoma is required regarding the prognostic value of ALT. Next, mechanistic studies are required to understand how ALT influences immune cell function through the TME of chondrosarcoma. Future research should investigate potential therapeutic strategies targeting ALT pathways and explore the role of metabolic reprogramming following the treatment.

Conclusions

This study redefines the role of ALT in chondrosarcoma, challenging the conventional view that ALT activation is typically associated with poor prognosis in sarcomas. Our findings demonstrate that ALT-like chondrosarcomas exhibit significantly improved survival compared to non-ALT-like tumors, highlighting a distinct metabolic and immune landscape. We suggest a set of biomarkers (SIG100) that reliably distinguishes ALT-like from non-ALT-like chondrosarcomas, providing a valuable prognostic tool and a potential avenue for personalized therapeutic strategies. Collectively, these findings establish ALT status as a key determinant of chondrosarcoma progression and expand opportunities

for targeted interventions based on tumor-specific TMM profiles.

Abbreviations

ALT	Alternative lengthening of telomere
KEGG	Kyoto Encyclopedia of Genes and Genomes
LIHC	Liver hepatocellular carcinoma
NK	Natural killer
TCGA	The Cancer Genome Atlas
TMM	Telomere maintenance mechanism

Author contributions

Conceptualization, J.Y.S.; methodology, J.Y.S.; formal analysis, J.Y.S.; writing—original draft, J.Y.S.; writing—review and editing, J.Y.S., J.H.K., Y.J.K; supervision, J.H.K., Y.J.K.; funding acquisition, J.H.K, Y.J.K.; All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (No. RS-2023-00213119, RS-2024-00440273, RS-2025-00517430), and the National R&D Program for Cancer Control, Ministry of Health and Welfare, Republic of Korea (RS-2024-00332838).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, South Korea. [2]Department of Environmental Medicine, College of Medicine, Ewha Womans University, Seoul 07804, South Korea. [3]Department of Radiation Oncology, College of Medicine, Ewha Womans University, Seoul 07804, South Korea.

Received: 26 March 2025 Accepted: 25 April 2025
Published online: 12 May 2025

References

1. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016;8:69.
2. De Vitis M, Berardinelli F, Sgura A. Telomere length maintenance in cancer: at the crossroad between telomerase and alternative lengthening of telomeres (ALT). Int J Mol Sci. 2018;19:606.
3. Cesare AJ, Reddel RR. Alternative lengthening of telomeres: models, mechanisms and implications. Nat Rev Genet. 2010;11(5):319–30.
4. Mori JO, Keegan J, Flynn RL, Heaphy CM. Alternative lengthening of telomeres: mechanism and the pathogenesis of cancer. J Clin Pathol. 2024;77:82–6.
5. Palmisciano P, et al. Primary skull base chondrosarcomas: a systematic review. Cancers (Basel). 2021;13(23):5960.
6. Ravindran PK, et al. Skull-base chondrosarcoma: a systematic review of the role of postoperative radiotherapy. Cancers (Basel). 2024;16(5):856.
7. Sung J-Y, Lim H-W, Joung J-G, Park W-Y. Pan-cancer analysis of alternative lengthening of telomere activity. Cancers (Basel). 2020;12:2207.
8. Barthel FP, Wei W, Tang M, Martinez-Ledesma E, Hu X, Amin SB, et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. Nat Genet. 2017;49:349–57.
9. Lawlor RT, et al. Alternative lengthening of telomeres (ALT) influences survival in soft tissue sarcomas: a systematic review with meta-analysis. BMC Cancer. 2019;19(1):232.
10. Sung J-Y, Cheong J-H. Pan-cancer analysis of clinical relevance via telomere maintenance mechanism. Int J Mol Sci. 2021;22:11101.
11. Hu J, Hwang SS, Liesa M, Gan B, Sahin E, Jaskelioff M, et al. Antitelomerase therapy provokes ALT and mitochondrial adaptive mechanisms in cancer. Cell. 2012;148:651–63.
12. Sung J-Y, Cheong J-H. Alternative lengthening of telomeres is mechanistically linked to potential therapeutic vulnerability in the stem-like subtype of gastric cancer. Clin Transl Med. 2021;11: e561.
13. Flynn RL, Cox KE, Jeitany M, Wakimoto H, Bryll AR, Ganem NJ, et al. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. Science. 2015;347:273–7.
14. Hakin-Smith V, Jellinek D, Levy D, Carroll T, Teo M, Timperley W, et al. Alternative lengthening of telomeres and survival in patients with glioblastoma multiforme. Lancet. 2003;361:836–8.
15. Hao Y, Stuart T, Kowalski MH, Choudhary S, Hoffman P, Hartman A, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nat Biotechnol. 2024;42:293–304.
16. Su Z, et al. A single-cell atlas of conventional central chondrosarcoma reveals the role of endoplasmic reticulum stress in malignant transformation. Commun Biol. 2024;7(1):124.
17. Grün D, Muraro MJ, Boisset J-C, Wiebrands K, Lyubimova A, Dharmadhikari G, et al. De novo prediction of stem cell identity using single-cell transcriptome data. Cell Stem Cell. 2016;19:266–77.
18. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan C-H, et al. Inference and analysis of cell-cell communication using Cell Chat. Nat Commun. 2021;12:1088.
19. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47(W1):W556–60.
20. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun. 2019;10:1523.
21. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinfor. 2013;14:7.
22. Sung J-Y, Cheong J-H. Pan-cancer analysis reveals distinct metabolic reprogramming in different epithelial-mesenchymal transition activity states. Cancers (Basel). 2021;13:1778.
23. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
24. Sieverling L, Hong C, Koser SD, Ginsbach P, Kleinheinz K, Hutter B, et al. Genomic footprints of activated telomere maintenance mechanisms in cancer. Nat Commun. 2020;11:733.
25. Sharaf R, Jin DX, Grady J, Napier C, Ebot E, Frampton GM, et al. A pan-sarcoma landscape of telomeric content shows that alterations in RAD51B and GID4 are associated with higher telomeric content. NPJ Genom Med. 2023;8:26.
26. Bovee JVMG, van den Broek LJCM, Cleton-Jansen A-M, Hogendoorn PCW. Chondrosarcoma is not characterized by detectable telomerase activity. J Pathol. 2001;193:354–60.
27. Sung J-Y, Lee JW. Telomere maintenance mechanism subtype reveals different immune activity in vestibular schwannoma. J Neurooncol. 2023;165:113–26.

Publisher's Note