The c-Myc/TBX3 axis promotes cellular transformation of sarcoma-initiating cells

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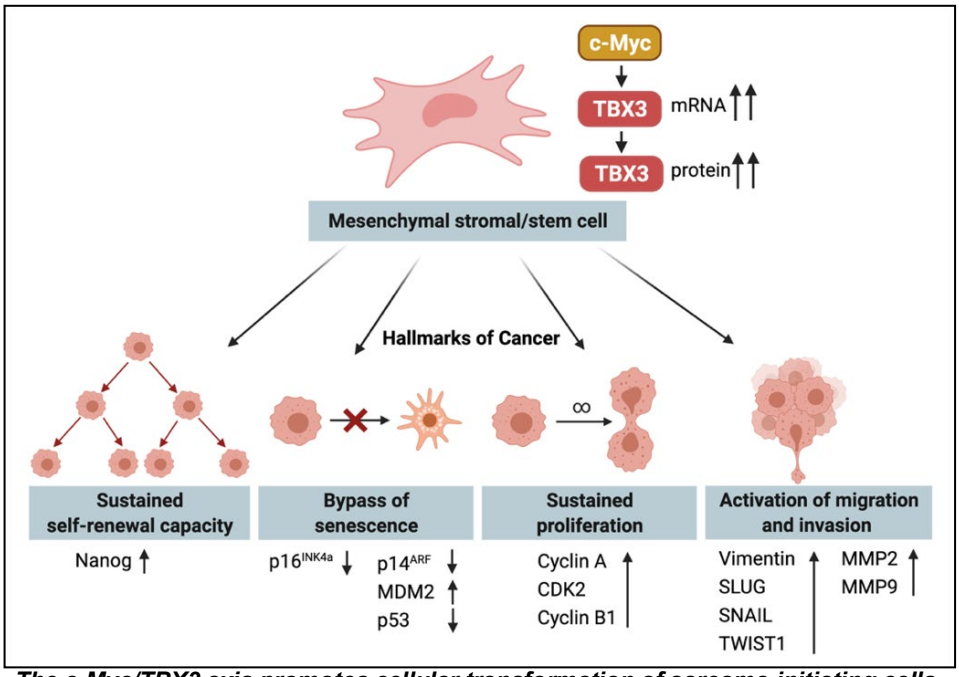

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Introduction: Sarcomas are highly aggressive cancers of mesenchymal origin whose clinical management is highly complex. This is partly due to a lack of understanding of the molecular mechanisms underpinning the transformation of mesenchymal stromal/stem cells (MSCs) which are presumed to be the sarcoma-initiating cells[1]. c-Myc is amplified/overexpressed in a range of sarcomas where it has an established oncogenic role and there is evidence that it contributes to the malignant transformation of MSCs. T-box transcription factor 3 (TBX3) is upregulated by c-Myc in a host of sarcoma subtypes where it promotes proliferation, tumor formation, migration, and invasion[2,3]. This study investigated whether TBX3 is a c-Myc target in human MSCs (hMSCs) and whether overexpressing TBX3 in hMSCs can phenocopy c-Myc overexpression to promote malignant transformation.

Methodology: Whether c-Myc regulates TBX3 in hMSCs was examined using siRNA, qRT-PCR, luciferase reporter and chromatin-immunoprecipitation assays. FLAG-TBX3 overexpressing hMSCs were generated using lentiviral genetransfer and the impact of TBX3 overexpression was investigated in 2D- and 3D-cell culture models. These included colony formation unit assays, senescence-associated β-galactosidase staining, growth curves, calcein AM staining, 2D-scratch motility assays, 3D-collagen invasion assays and global gene expression profiling by microarray analysis and gene set enrichment analysis.

Results: We show that c-Myc transcriptionally activates TBX3 through a conserved E-box motif in hMSCs and that TBX3 mediates the pro-proliferative ability of c-Myc. TBX3 overexpression in hMSCs promoted self-renewal, bypass of senescence and enhanced proliferation. This was associated with an increase in NANOG, a decrease in p16INK4a, increased levels of cell cycle progression markers (cyclin A, CDK2, cyclin B1) and downregulation of the $p14^{ARF}$/MDM2/p53 tumor suppressor pathway. Furthermore, TBX3 promoted hMSCs migration and invasion and increased markers of migration (vimentin, SLUG, SNAIL, TWIST1) and invasion (MMP2, MMP9). Genes upregulated upon TBX3 overexpression were involved in cell cycle progression and cell division, overlapped with c-Myc targets, and are associated with sarcomagenesis.

Discussion and Conclusions: The c-Myc/TBX3 oncogenic molecular pathway may be a key mechanism that transforms hMSCs into sarcomas.

References:

[1]Damerell V, Pepper MS, Prince S. Molecular mechanisms underpinning sarcomas and implications for current and future therapy. *Signal Transduct Target Ther* (2021) 6:246.
[2]Willmer T, Peres J, Mowla S, Abrahams A, Prince S. The T-box factor TBX3 is important in S-phase and is regulated by c-MYC and cyclin A-CDK2. *Cell Cycle* (2015) 14:3173-3183.
[3]Willmer T, Cooper A, Sims D, Govender D, Prince S. The T-box transcription factor 3 is a promising biomarker and a key regulator of the oncogenic phenotype of a diverse range of sarcoma subtypes. *Oncogenesis* (2016) 5:e199.

Keywords: Mesenchymal stromal/stem cells (MSCs), sarcoma, c-Myc, TBX3, tumorigenesis, spheroids