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Molecular genetic biomarkers: Chromosome 1 and 19 abnormalities in glioblastoma are associated with adverse molecular features and overall reduced survival

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Introduction: Molecular genetic alterations are constantly being reported in malignant brain tumors and play a significant role in diagnosis, prognosis, and management. Glioblastoma (GB) is defined by diffuse infiltrative growth with nuclear atypia, IDH wildtype and either: mitotic activity, necrosis, or microvascular proliferation or molecular TERT promoter mutation or EGFR gene amplification or chromosome 7 and chromosome 10 loss. The molecular features of TERT promotor mutation have been found to be associated with poor prognosis in patients with GB while MGMT promotor methylation and EGFR gene amplification are associated with favorable prognosis. Furthermore, CDKN2A deletions have been correlated with unfavorable features and worse prognosis in gliomas and in GB. On the contrary, deletions on chromosomes 1p and 19q in oligodendrogliomas and CNS WHO grade II-III glioma have been shown to correlate with improved prognosis and overall survival largely due to the increased chemosensitivity of these tumors. However, in GB, the impact of these alterations has yet to be elucidated. Therefore, illuminating the impact of these chromosomal abnormalities in GBM could potentially improve current strategies for patient prognosis and management.
Methods: The purpose of this study was to identify patients with GB who had chromosome 1 and/or 19 alterations and correlate this result with other molecular genetic features and overall survival. Hence, we retrospectively evaluated 90 brain tumors from 2018 to February 2023 on whom fluorescence in situ hybridization (FISH) for chromosome 1p and 19q was performed. We correlated the FISH results with clinical, histopathological and other molecular genetic findings. Fisher's exact test was used to calculate statistical significance.
Results: Of the 90 tumors evaluated, 42 tumors were classified as GB. 16 (38%) of the GB had chromosome 1/chromosome 19 abnormalities and 26 (62 %) did not have these chromosomal abnormalities. Of the 16 GBM with chromosome 1/19 alterations, 4/16 (25%) had chromosome 1p loss, 4/16 (25%) had polysomy 1, 2/16 (12.5%) had chromosome 19q deletion, 4/16 (25%) had chromosome 19q gain, and 8/16 (50%) had polysomy 19. Compared to the control group (GBM without chromosome 1/19 abnormalities), the study group (GB with chromosome 1/19 abnormalities) had higher associations with adverse molecular features (CDKN2A deletions, TERT promoter mutations), similar associations with favorable molecular features (MGMT promoter methylation, EGFR gene amplification) and overall reduced survival ($P = 0.01$, Statistical significance was calculated using Fisher's Exact Test).
Conclusion: The incidence of adverse prognostic molecular features and overall reduced survival are significantly higher in GBM with chromosome 1 and chromosome 19 abnormalities compared to those without these chromosomal abnormalities. In contrast to oligodendrogliomas and WHO grade II-III gliomas, chromosome 1p and 19q deviations were found to be associated with reduced survival in patients with GBM. The findings of this research have the potential for improved prognostic information for patients with GBM and highlights the value of molecular genetics in diagnosis, prognosis, and treatment of gliomas.

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Single-cell and spatial transcriptomics integrated with bulk RNA-seq, uncovers differences in bidirectional tumor-macrophage crosstalk in IDHwt and IDHmut gliomas

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Introduction: Gliomas are the most aggressive form of brain tumors. Despite notable advancements in targeted and immunotherapies, the standard of care for glioblastoma (GBM) has remained largely unchanged, predominantly due to the challenges posed by the intra-tumoral heterogeneity and its immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs) residing in the TME are characterized by their pivotal roles in tumor progression, antitumor immunity and TME remodeling. However, knowledge of tumor-macrophage crosstalk in major categories of gliomas remains elusive. In the current study, tumor-TAM crosstalk in IDHwt and IDHmut gliomas was further explored making use of single-cell and spatial transcriptomics to elucidate intricate mechanisms leading to aggressive phenotype of GBM.
Methods: Single cell RNA-seq was used to dissect the heterogeneity of TAMs in IDHwt (N=5) and IDHmut (N=5) tumors. Gene set activity assessment using AUCell package combined with the expression of canonical biomarkers was carried out to annotate myeloid and microglial subsets. Afterwards, spatial distribution of annotated subpopulations was resolved by spatial transcriptomic data. Next, Differentially Expressed Genes (DEGs) and their enriched pathways were investigated between IDHwt and IDHmut bone-marrow and microglial (MG) derived TAMs. Palantir and CellRank2 were utilized to conduct trajectory inference analysis and TAMs were ordered along a high resolution pseudotime. Using SCISSOR R package, survival and genomic information provided by the TCGA reference with cell-type signatures of our scRNA-seq dataset were integrated. pySCENIC was used to infer gene regulatory networks that govern TME subpopulations followed by snATAC-seq analysis to assess cell-state specific chromatin accessibility of identified regulons. CellPhoneDB V3.0 was applied to study cell-cell communications in IDHwt and IDHmut tumors followed by spatial transcriptomics, to further investigate spatial communication of inferred tumor-TAM interplay. A comparison of the expression of ANXA1, NRP1, FN1 and TNFRSF12A genes between bulk RNA-seq profiles of TCGA-GBM (N=162) and TCGA-LGG (N=534) cohorts was made using DESeq2 R package for statistical analysis. Subsequently, GLASS bulk RNA-seq was used for longitudinal expression analysis of candidate genes. In order to validate bulk RNA-seq results, Real-time qPCR on IDHwt (N=24) and IDHmut (N=24) fresh samples was performed. The samples were surgically resected specimens collected from the Neurosurgery ward of Shariati hospital affiliated with Tehran University of Medical Sciences (TUMS).

Results: As a result, heterogeneity of TAMs was explained by six subpopulations of MG-TAM, MG-TAM.AGING, MG-TAM.PROLIF, BMD-TAM.MES, BMD-TAM.MES/HYPO and BMD-TAM.Anti.INF. Differential expression analysis revealed that IDHwt BMD-TAMs were enriched for genes implicated in Oxidative phosphorylation and hypoxia-response mechanisms whereas Interferon Gamma and Alpha responses were the two most enriched pathways in IDHwt MG-TAMs. Two subpopulations of BMD-TAM.MES and BMD-TAM.MES/HYPO, composed of larger proportions of TME in IDHwt, displayed the most prominent association with worse survival. Inference of gene regulatory networks identified ELK1 transcription factor highly enriched in these subclusters. snATAC-seq confirmed differential accessibility of ELK1 binding motifs in BMD-TAM.MES and BMD-TAM.MES/HYPO cells. Cell-cell communication analysis identified IDHwt and IDHmut-unique ligand-receptor combinations. ANXA1-FPR1/3, VEGFA-NRP1, FN1-integrin and TWEAK-FN14 were the IDHwt-only combinations opted for further analysis. In validation of TCGA bulk RNA-seq findings, qRT-PCR results presented *ANXA1*, *NRP1*, *FN1* and *FN14* genes highly overexpressed in IDHwt GBM compared to IDHmut samples. Longitudinal expression follow-up exhibited no significant difference between paired initial and recurrent tumors.
Conclusion: Our results suggest that aggressive properties of IDHwt GBM can be elucidated in part by the unique communications they form with their associated macrophages in TME.

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Transgenic lines to investigate the impact of IL7Rα gain-of-function mutation during hematopoietic development in zebrafish embryos

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Introduction: Acute lymphoblastic leukemia (ALL) is the most prevalent childhood malignancy, with a subset of cases characterized by uncontrolled expansion of T-lymphoid cells (T-ALL). The IL-7R pathway plays a crucial role in T-cell maturation and development, with approximately 25% of T-ALL cases associated with aberrant IL-7R signaling. Gain-of-function mutations in IL-7Rα have been first identified and described in pediatric T-ALL patients at Boldrini Children's Center. Zebrafish models have emerged as valuable tools for cancer research, as hematopoiesis and signaling pathways involved in this process are highly conserved between zebrafish and humans. In this project, we aim to generate transgenic zebrafish lines expressing the human IL-7Rα gain-of-function mutation (hIL-7Rαmut) to study its biological function during T-cell development and maturation.
Methods: The hIL7Rαmut and promoter sequences were amplified using PCR and inserted into the pT2AL200R150G plasmid via In-fusion reaction. This plasmid contains the constitutive promoter Ef1α, transposable elements for genomic integration (tol2 system), and green fluorescent protein (GFP) fused with the mutation. The constitutive promoter will be replaced with cell-specific promoters (zC-myb for hematopoietic stem cells, zRag2 for lymphocyte precursors, and zLCK for mature T lymphocytes). The cloned constructs were validated and injected into 1-cell stage AB zebrafish embryos, along with transposase mRNA. Embryos were collected at different developmental stages for molecular analyses, in vivo imaging, phenotype characterization, and generation analyses.
Results: Two constructs containing the mutation (Ef1α:hIL7rαmut:GFP and zCmyb:hIL7Rαmut:GFP) were successfully cloned and sequenced, confirming the presence of the mutation and promoter sequence. Luciferase assays in transfected HEK-293T cells demonstrated that the fusion to GFP does not interfere with receptor activity. Injection of the constructs into zebrafish embryos resulted in fluorescent receptor expression in various cell types and throughout the body (Ef1α:hIL7Rmut:GFP), as well as delimited expression in the caudal hematopoietic tissue (CHT) where hematopoietic stem cells reside (zC-myb:-hIL7rαmut:GFP). Genotyping confirmed the insertion of the mutation in the genome. The injected animals (F0 generation) are mosaic and will be further analyzed and maintained for future crossings and lineage establishment. Gene expression analyses will be conducted to assess the impact of the mutation on hematopoietic development.
Conclusion: Validated constructs have been generated to produce transgenic zebrafish carrying the hIL7Rα mutation without affecting receptor activity. These models provide a valuable platform to investigate the role of IL7Rα gain-of-function mutation in T-cell development. The observed phenotypes and genotypic confirmation lay the foundation for further molecular analyses and observation of disease progression in these transgenic animals.

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Myelodysplastic syndrome (MDS) detected by germline genetic testing for hereditary cancer

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Background: Hereditary cancer genetic testing using massively parallel sequencing can reveal incidental findings such as mosaicism, clonal hematopoiesis, or hematologic malignancies. We report a case of a patient who had germline genetic testing which revealed a contiguous gene deletion of the long arm of chromosome 5 at low allele fraction, leading to a diagnosis of myelodysplastic syndrome (MDS).
Case presentation: The patient is a 68-year-old male with Ewing's sarcoma at age 16, male breast cancer at age 53, and papillary thyroid cancer at age 57. He has a family history of young breast cancer, prostate cancer, oligodendroglioma, glioblastoma and pituitary adenoma. The patient had next-generation sequencing multi-cancer panel testing for 76 genes completed using a blood sample. This initial test revealed three heterozygous variants of uncertain significance (VUSs) in *POT1, MLH3* and *ATM*, as well as whole gene deletions of both *APC* and *CTNNA1* at low allele fraction (~20%). The *APC* and *CTNNA1* genes are located on the long arm of chromosome 5 (5q) approximately 26 Mb apart, which suggested that these deletions may reflect a contiguous gene deletion. These results therefore raised a few possible differential diagnoses: mosaicism, a somatic hematologic event such as clonal hematopoiesis, or a hematologic malignancy. To further investigate the origin and significance of the deletion, the patient underwent a skin biopsy and had the multi-cancer panel repeated on cultured fibroblasts. Fibroblast testing did not reveal the *APC* or *CTNNA1* deletions, nor did it reveal the VUS in *ATM*, suggesting these variants