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The Pilot Study of C-jun and TGF-beta Immunoexpression in Relation to the Oldest Cancer Biomarkers – Tumor Histology and Proliferation Rate in Glioblastoma and Neuroblastoma Models of Cancer Disease

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Abstract

Background: *Prognostic factors in cancers are believed to be one of the most important discoveries in oncology. However, due to the development of integrated science they are not restricted to morphological anomalies anymore. In everyday routine the light microscope markers are systematically replaced by new ones, especially of immunohistochemical and molecular type. It leads to the question what is the relation of the oldest prognostic parameters to the basic and newly discovered pathways common for cancer disease itself.*

Objectives: *The aim of presented studies is to investigate the most modern factors involved in pathological processes in the two models of cancer disease, glioblastoma in adults and neuroblastoma in developmental age to search for potential relation with the established prognostic factors appropriately to the tumor type, including the oldest known light microscope parameters – tumor histology and proliferation rate of cancer cells.*

Material and methods: *Immunohistochemical assessment of expression of c-jun in glioblastoma and TGF-beta in neuroblastoma group in relation to chosen histoclinical features: patients' related (age, gender) and tumor related; including all the widely excepted prognostic parameters regarding the tumor type (location, histological type (neuroblastoma, ganglioneuroblastoma, ganglioneuroma), tumor histology (favorable, unfavorable), Ki-67 index, stage.*

Results: *Variations of c-jun immunoexpression were revealed in glioblastoma as well as differences in TGF-beta expression in neuroblastoma regarding the examined histoclinical features. Furthermore, in both cancer groups the levels of the examined protein expression appeared to relate to cancer cell proliferation estimated by the established parameter – Ki67 indices.*

Conclusions: *In both models of cancer disease, glioblastoma in adults and neuroblastoma in developmental age there is a crossing of pathways of the oldest and the newest cancer disease markers. Although integrated science offers the most advanced approaches it is important to consider the old*

established prognostic parameters in prognostication in individual, especially doubtful cases.

Key words: *biomarkers, intercellular signalling peptides and proteins, glioblastoma, neuroblastoma*

Introduction

Glioblastoma (GBM) is grade IV glioma according to WHO and one of the most aggressive neoplasms in humans, characterized by extremely poor prognosis [1]. Although for now several molecular markers are confirmed to be relevant in the development of glioblastoma (isocitrate dehydrogenase (IDH) mutations, MGMT promoter methylation, EGFR amplification/mutations, and vascular endothelial growth factor (VEGF) overexpression, the most characteristic and mandatory feature for the GBM diagnosis is light microscope finding – the proliferation of endothelial cells [2–5]. Angiogenesis in GBM makes this tumor a suitable model of cancer growth as well as highly investigated process for the knowledge of the neoplastic disease itself.. Angiogenesis in GBM makes this tumor a suitable model of cancer growth as well as highly investigated process for the knowledge of the neoplastic disease itself.

Neuroblastoma (NBL) may start in early forms of nerve cells found in a developing embryo and is typical cancer of infancy and early childhood. However, these tumors themselves present complexity of cancer disease from spontaneous regression to unjustified treatment failures and as the group make well known cancer disease model for developmental age [6]. They are also characterized by extremely numerous prognostic parameters: patient's age, tumor histology (favorable histology or unfavorable histology), DNA ploidy, MYCN gene amplifications, chromosome changes: 1p deletions, 11q deletions, 17q gain, Neurotrophin (nerve growth factor) receptors: especially TrkA, serum (blood) levels of ferritin and neuron-specific enolase (NSE) and lactate dehydrogenase (LDH). However, despite their high number of markers the course of the disease is unpredictable in all of the cases.

C-jun and TGF-beta proteins are modern and highly investigated proteins involved in pathological processes, especially cancer disease [7, 8]. It is underlined in both – they are fundamental part in basic pathways characterized cancer itself [9–11].

The role of the c-jun protein that belongs to AP-1 family is to signal--transduce transcription which make it a crucial factor in the cell life cycle [12–14].

TGFβ is believed to be an extremely important factor in the process of tumorigenesis and invasiveness by being involved in evoking oxidative stress that is associated with cancer development [15].

The aim of presented studies was to investigate the expression of above listed factors in two models of cancer disease – glioblastoma in adults and neuroblastoma in developmental age to search for their potential relation with the established prognostic factors appropriately to the tumor type, including the oldest known light microscope parameters - tumor histology and proliferation rate of cancer cells.

Material and methods

The studies were performed according to the agreement of Bioethics Committee of the Medical University od Lodz RNN/350/19/KE and RNN/244/19. Each cancer study group consisted of 20 cases from the archives of Department of Pathomorphology, Chair of Oncology, Medical University of Lodz, Poland. Glioblastoma and neuroblastoma tissue samples were paraffin embedded and these paraffin blocks were sectioned to 4–5 μm samples, routinely stained with hematoxylin and eosin (H+E) and used for further immunohistochemical tests in the PowerVision detection system by ImmunoLogic according to the immunoperoxidase method, with the use of primary rabbit polyclonal antibodies directed against c-jun by Cell Signalling Technology and TGFβ by Biorbyt. Paraffin sections placed on adhesive slides and dried in an incubator at 56°C for 1 hour, were passed to dewaxing in a series of xylenes and alcohols of decreasing concentrations (96%, 80%, 70%, 60%). By the use of 3% solution of perhydrol in methanol for 5 minutes endogenous peroxidase activity could be inhibited. For the purposes of immunohistochemistry, it was required to preserve tissue antigenicity and made it proper for the examined antibodies to work. In order to do that a specific sequence had to

be followed. The sections were heated in Target Retrieval Solution at pH 9.0 DAKO, in a water bath at 95°C for 45 min. After cooling, they were washed twice in 0.05 M TRIS buffer (TBS) at pH 7.6 for 5 min. Then they were subjected to overnight incubation in a refrigerator at 4°C, in a humid chamber, with appropriately diluted antibodies: anti-c-jun 1:50 and anti-TGFβ 1:400.

The next step was to wash it twice in TBS and visualize in the ImmunoLogic PowerVision two-step visualization system to observe the antigen-antibody response. In order to do that the sections were incubated 30-minute in the above-mentioned conditions with a polymer labelled with peroxidase and associated with secondary goat antibodies directed against the used polyclonal rabbit antibodies. The last stage of detection was an enzymatic reaction in which a colored product appeared when the substrate for peroxidase – 3,3` –diaminobenzidine tetrachloride (DAB) was used (incubation time with DAB solution – 2 min.). In the end, when a positive immunohistochemical reaction was achieved, the nuclei were stained with Meyer's hematoxylin (2 min.), and then the sections were dehydrated in a series of alcohols of increasing concentrations (70%, 80%, 96%) and passed through a series of acetones and xylenes. The prepared Histokit was embedded in an anhydrous medium. The negative control of the method were sections in which the primary antibodies were replaced with TBS using the same immunohistochemical procedure. Sections showing a previously known strong positive reaction to the test antigen were used as a positive control. The slides were assessed using a light microscope. Positive reaction was defined as the brown coloration of cell nuclei and presented as an index in each case. Histoclinical analysis of c-jun or tgf-beta expression was done regarding patients' related features (age, gender), tumor related parameters including all the widely excepted prognostic parameters regarding the tumor type (location, histological type – neuroblastoma, ganglioneuroblastoma, ganglioneuroma, tumor histology – favorable or unfavorable), Ki-67 index, stage, lymph node status, distant metastases, recurrences). The obtained data were evaluated in statistical analysis

that was performed with the use of the statistical packages rstatix and ggpubr in the R environment (R 4.0.2). In the first stage, the Shapiro--Wilk test followed by Levene's test were carried out and in the subsequent stages of the analysis, non-parametric Kruskal-Wallis test was used. The hypotheses were tested by applying a non-parametric signed rank Wilcox test or in the case of normally distributed data the t-test. Correlations between parameters were calculated using the Spearman coefficient. The significance level (p) was considered statistically significant when its value was < 0.05.

Results

Glioblastoma study

C-jun immunoexpression was found in all the examined GBM cases and appeared variously with differences in gender, age, and location of the tumor as well as Ki-67 indices (Figure 1).

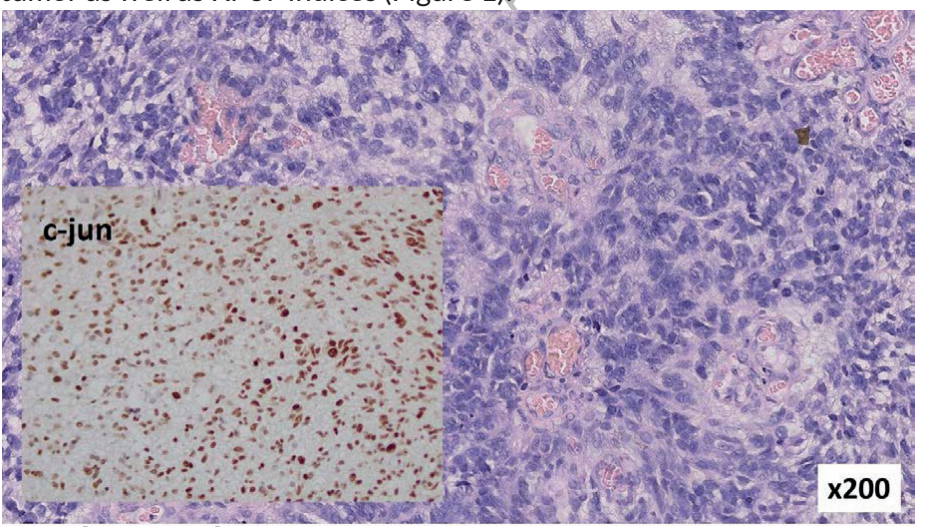

Figure 1. The example of c-jun immunoexpression in GBM (left), the background – proliferation of endothelial cells in GBM, hematoxylin and eosin (H&E) stain, orig. magn. 200x

The c-jun indices appeared as follow:

Among males, the median c-jun expression was 38% (mean = 36.7 ± 17.3), whereas among females it was lower and reached 34% (mean = 32.6 ± 7.57) (Figure 2).

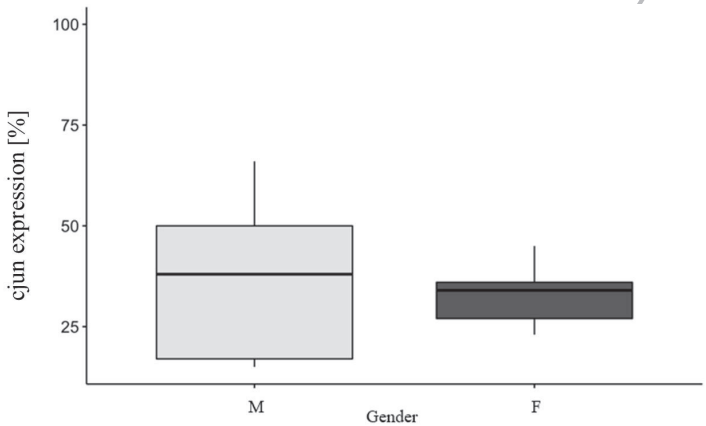

Figure 2. The box-plot of the values of difference of c-jun expression between males (M) and females (F) in GBM group

Median c-jun expression for right hemisphere, left hemisphere, and brain in general was 40.5% (mean = 38.3 ± 16.2), 39.5% (mean=36 ± 15.5), and 32% (mean = 33.1 ± 14.4), accordingly (Figure 3). Differences in c-jun immunoexpression indices listed above do not appear statistically significant.

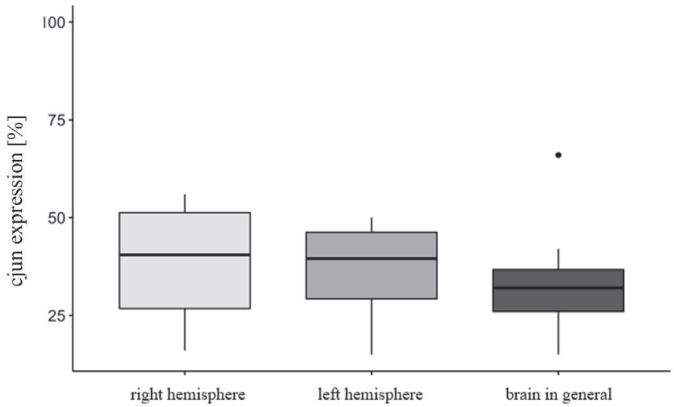

Figure 3. The box-plot of the values of difference of c-jun expression regarding tumor location in GBM group

There was observed a moderate association of c-jun expression and age of women (rho=-0.64) (Figure 4), a strong association of c-jun expression and age and location in left hemisphere (rho = -0.80) (Figure 5), as well as c-jun expression and Ki-67 expression in right hemisphere located tumors (rho = -0.71) (Figure 6).

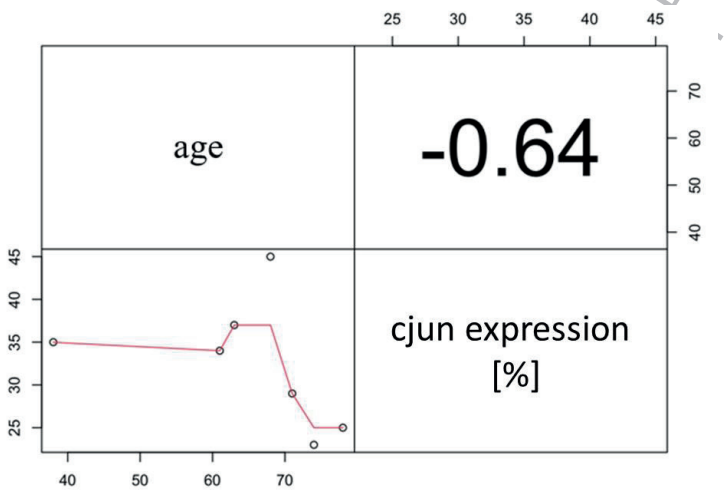

Figure 4. Correlation between c-jun expression and patients age among females – rho Spearman

Figure 5. Correlation between c-jun expression, patients age and tumor location in left hemisphere – rho Spearman

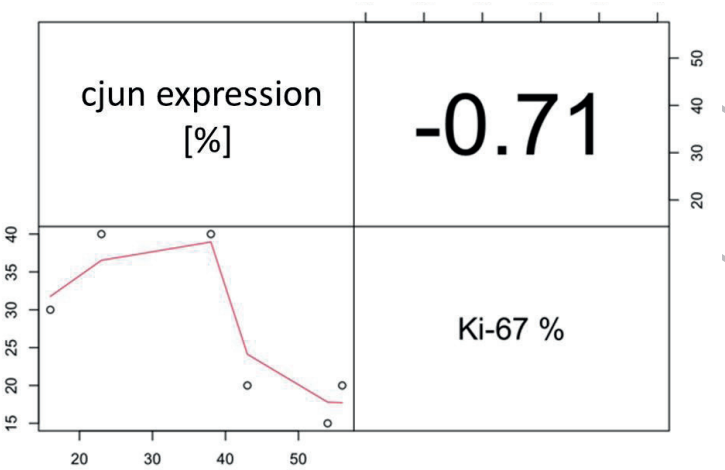

Figure 6. Correlation between c-jun expression, Ki-67 expression and tumor location in right hemisphere – rho Spearman

Neuroblastoma group

TGF-beta immunoexpression was found in all the examined GBM cases and appeared various with differences in gender, age, histological type (neuroblastoma, gangioneuroblastoma or ganglioneuroma), tumor histology (favorable or unfavorable) (Figure 7) .

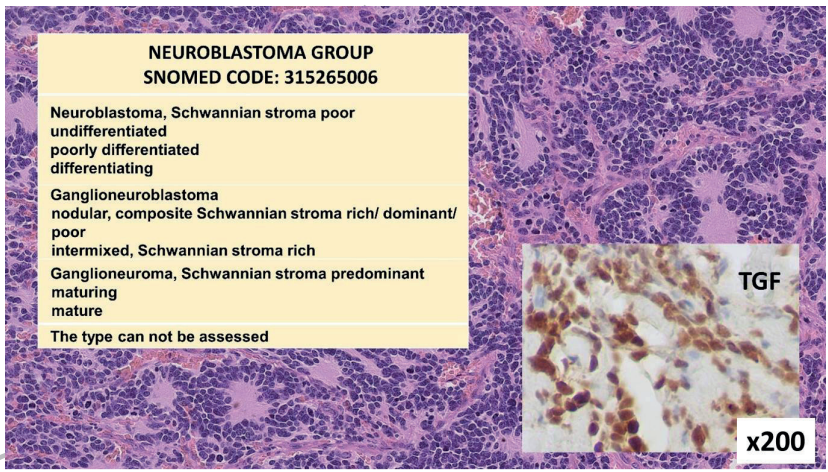

Figure 7. The example of TGF-beta immunoexpression in NBL (right), the background –Schwannian stroma poor, poorly differentiated NBL, hematoxylin and eosin (H&E) stain, orig. magn. 200x

There was revealed statistically significant relation of TGFβ expression in histological types of tumors of neuroblastoma group, p = 0.0024, including tumor histology (favorable and unfavorable), p = 0.0076 (Figure 8).

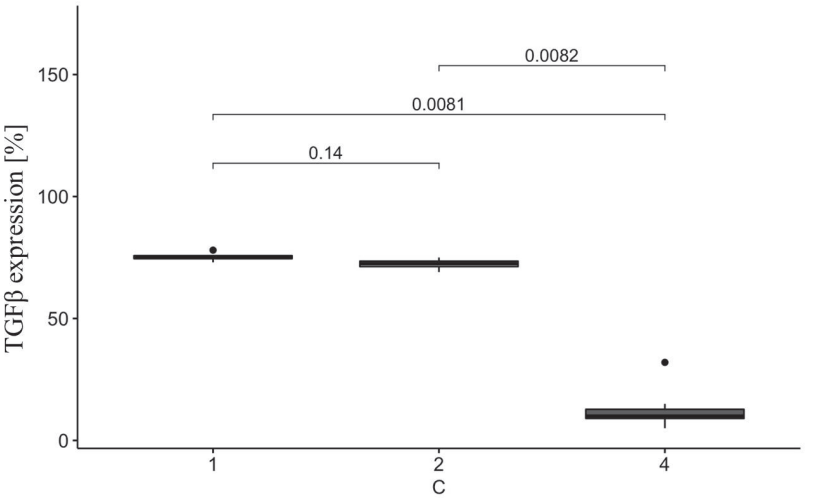

Figure 8. The values of TGFβ expression in NBL group (C) – C1 neuroblastoma, C2 ganglioneuroblastoma, C3 ganglioneuroma

Discussion

The search for universal cancer biomarkers is one of the leading aims of current oncology. Complete understanding of basic pathways of cancer disease seems to be the key to cancer treatment and creating the personalized strategies that will allow to achieve optimal results. Various patterns of c-jun expression in glioblastoma and TGF-beta in neuroblastoma group obtained in presented studies are understandable regarding the fact that both the examined cancers cover the fundamental cancer mechanisms, becoming the models for cancer disease in adulthood and developmental age, appropriately.

C-jun expression is associated with the majority of the occurring processes including proliferation, differentiation and apoptosis [12]. Its up-regulation is commonly seen in the central nervous system and evidences show its connection with many pathologies such as inflammations,

ischemia and neurodegenerative diseases as well as with many physiological reparative processes [13, 14, 16]. It is also noticeable that many studies confirm its involvement in development and progression of human malignancies including glioblastoma [7, 8, 17–19].

Although, the obtained results did not achieve the minimum threshold of statistical significance probably due to the limited study subgroups, where only huge differences within homogeneous groups may be proven, they well illustrate the complexity of GBM biology with non-complex morphology and lack of prognostic significance of morphological findings.

There are very intriguing observed differences in c-jun expression in GBM of the right and left hemisphere, that seem to be worth of further evaluation. Among these findings it is important to underline the correlation of c-jun and Ki-67 expression found in the right hemisphere. The estimation of cancer cell proliferation is one of the oldest prognostic parameters with widely accepted value in many cancer types. Preliminary attempts of investigation of proliferation rate were based on light microscope assessment of mitotic figures, however with the development of scientific tools they were successfully replaced by more objective immunohistochemical stains with the use of universal Ki-67 evaluation. In addition, modern immunohistochemistry allows to evaluate the proliferating activity of cells much more precisely. Its importance is also confirmed in some entities in which proliferation rate assessment is not included in routine protocols [20]. Regarding the obtained results the crossing of that basic cancer mechanisms and c-jun expression pathways what stay in compliance with the literature may be suggested [19].

Very characteristic feature of neuroblastoma group is its heterogeneity what have an influence on prognosis and treatment that have to be adjusted personally to a patient. The knowledge about all of the prognostic factors that are associated with the development of neuroblastoma are crucial for assessing the patient's survival. Numerous prognostic parameters in NBL underline complexity of this disease and unknown pathways of NBL biology [21, 22].

TGFβ is produced by both immune and non-immune cells and it has a very important role in functioning of many biological processes such as embryogenesis, immunological processes and carcinogenesis [23]. TGFβ is believed to be involved in tumorigenesis and invasiveness by being a contributor in oxidative stress that is associated with cancer development [15].

Neuroblastoma is a common childhood neoplasm and the death in the patients with this disease is caused mostly by the development of metastasis. TGFβ level was proved to be increased in neuroblastoma cases and staying in connection with the patient's prognosis [24]. TGFβ as a component of ERK1/2 and TGFβ1 signaling pathways was analyzed in the patients with neuroblastoma as the potential regulator of proliferation, migration and invasion of human neuroblastoma cells. It was considered as a crucial factor in the process of cancer development and a potential therapeutic target [25].

It was revealed in presented studies that TGFβ, what stays in compliance with literature, is well known and powerful inhibitor of cell proliferation and a potent inducer of differentiation. Resistance to its action characterized many malignancies and has been attributed to alterations of its pathways and receptors, either [26]. Transforming growth factor (TGF)-β level was indeed elevated in neuroblastoma cases and found to be related to a pathway important for promoting neuroblastoma invasion and tumor metastases has been shown to be associated with the epithelial-mesenchymal transition [27, 28].

The expression of TGFβ1 was evaluated in 51 primary tumors and 17 invasion/metastasis in Wilms' tumor cells. The higher level of TGFβ1 in primary Wilms' tumor was correlated with tumor invasion and disease progression and could be a potential prognostic and predictive factor [11]. Wilms' tumor belongs to group of embryonal tumors in which development nephrogenic rests have a crucial function. TGFβ plays a critical role in embryo development and is strictly associated with cellular proliferation and its growth. It is evidenced that TGFβ has the influence on Wilms' tumor development but the correlation between them is still not fully explained.

It was evidenced that overexpression of TGFβ1 was associated with stage of the disease and presence of invasion and metastasis [29]. The same the expression of TGFβ was checked in medulloblastoma by immunohistochemistry methods in order to be associated with its metastasis and patient's survival [30].

Final study finding which is the relation of TGF-beta expression in NBL to tumor histology needs reflection on proliferation rate involvement similarly to presented glioblastoma results. Apoptotic-mitotic index included in division into favorable and unfavorable histology of NBL probably influence the obtained results.

Concluding remarks. No matter what is the way of estimation, mitotic activity is a very good indicator of the proliferation potential which reflects fundamental cancer mechanism as well as it is crossed with newly discovered pathways what regarding the obtained results found in both the examined models may be a universal finding in cancer. Although integrated science offers the most advanced approaches, it is important when drill down for molecular or even atomic biomarkers to consider the old established prognostic parameters in prognostication in individual, especially doubtful cases [21, 31].

References

1. Louis DN, Perry A, Reifenberger G et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016; 131: 803–820.

2. Christians A, Adel-Horowski A, Banan R et al. The prognostic role of IDH mutations in homogeneously treated patients with anaplastic astrocytomas and glioblastomas. Acta Neuropathol Commun 2019; 7: 156.

3. Saadeh FS, Mahfouz R, Assi HI. EGFR as a clinical marker in glioblastomas and other gliomas. Int J Biol Markers 2018; 33: 22–32.

4. Wick W, Weller M, van den Bent M et al. MGMT testing – the challenges for biomarker-based glioma treatment. Nat Rev Neurol 2014; 10: 372–385.

5. Birkó Z, Nagy B, Klekner Á, Virga J. Novel Molecular Markers in Glioblastoma – Benefits of Liquid Biopsy. IJMS 2020; 21: 7522.

6. Taran K, Kobos J, Sitkiewicz A. Estimation of prognostic value of CD44 expression in neuroblastic tumours in children. Folia Neuropathologica 2007; 45: 126–132.

7. Schummer P, Kuphal S, Vardimon L, Bosserhoff AK, Kappelmann M. Specific c-Jun target genes in malignant melanoma. Cancer Biology & Therapy 2016; 17: 486–497.

8. Tsiambas E, Mastronikolis N, Fotiades PP, Kyrodi E. c-Jun/c-Fos complex in laryngeal squamous cell carcinoma 2020; 25: 618–620.

9. Tanyildiz HG, Kaygusuz G, Unal E, Tacyildiz N, Dincaslan H, Yavuz G. The prognostic importance of TGF-β, TGF-β receptor, and fascin in

childhood solid tumors. Pediatric Hematology and Oncology 2017; 34: 238–253.

10. Dai L, Gast A, Horska A et al. A case-control study of childhood acute lymphoblastic leukaemia and polymorphisms in the TGF-β and receptor genes. Pediatr Blood Cancer 2009; 52: 819–823.

11. Zhang L, Liu W, Qin Y, Wu R. Expression of TGF-β1 in Wilms' tumor was associated with invasiveness and disease progression. J Pediatr Urol 2014; 10: 962–968.

12. Bossy-Wetzel E. Induction of apoptosis by the transcription factor c--Jun. The EMBO Journal 1997; 16: 1695–709.

13. Raivich G. c-Jun expression, activation and function in neural cell death, inflammation and repair. Journal of Neurochemistry 2008: 107(4): 898–906.

14. Thakur A, Wang X, Siedlak SL, Perry G, Smith MA, Zhu X. c-Jun phosphorylation in Alzheimer disease. J Neurosci Res 2007; 85: 1668–1673.

15. Ramundo V, Giribaldi G, Aldieri E. Transforming Growth Factor-β and Oxidative Stress in Cancer: A Crosstalk in Driving Tumor Transformation. Cancers (Basel) 2021; 13: 3093.

16. Huang L, Quan X, Liu Z et al. c-Jun gene-modified Schwann cells: upregulating multiple neurotrophic factors and promoting neurite outgrowth. Tissue Eng Part A 2015; 21: 1409–1421.

17. Xiang D-M, Sun W, Zhou T et al. Oncofetal HLF transactivates c-Jun to promote hepatocellular carcinoma development and sorafenib resistance. Gut 2019; 68: 1858–1871.

18. Blau L, Knirsh R, Ben-Dror I et al. Aberrant expression of c-Jun in glioblastoma by internal ribosome entry site (IRES)-mediated translational activation. Proceedings of the National Academy of Sciences 2012; 109: E2875–2884.

19. Huang Z, Xia Y, Hu K, et al. Histone deacetylase 6 promotes growth of glioblastoma through the MKK7/JNK/c-Jun signaling pathway. J Neurochem 2020; 152: 221–234.

20. Taran K, Kobos J, Sporny S. Number of mitotic figures in microscopic picture of nephroblastoma histologic types-estimation of the value of the oldest known prognostic factor. Pol J Pathol 2008; 59: 43–47.

21. Coughlan D, Gianferante M, Lynch CF, Stevens JL, Harlan LC. Treatment and survival of childhood neuroblastoma: Evidence from a population-based study in the United States. Pediatr Hematol Oncol 2017; 34: 320–330.

22. Blessing MM, Alexandrescu S. Embryonal Tumors of the Central Nervous System: An Update. Surg Pathol Clin 2020; 13: 235–247.

23. Zhao H, Wei J, Sun J. Roles of TGF-β signaling pathway in tumor microenvirionment and cancer therapy. Int Immunopharmacol 2020; 89: 107101.

24. Shao J-B, Gao Z-M, Huang W-Y, Lu Z-B. The mechanism of epithelial--mesenchymal transition induced by TGF-β1 in neuroblastoma cells. International Journal of Oncology 2017; 50: 1623–1633.

25. Chen Z, Gao J, Sun J, Wu Z, Wang B. Aminoacylase 1 (ACY-1) Mediates the Proliferation and Migration of Neuroblastoma Cells in Humans Through the ERK/Transforming Growth Factor β (TGF-β) Signaling Pathways. Med Sci Monit 2021; 27: e928813.

26. Lolascon A, Giordani L, Borriello A et al. Reduced expression of transforming growth factor-beta receptor type III in high stage neuroblastomas. Br J Cancer 2000; 82: 1171–1176.

27. Micalizzi DS, Farabaugh SM, Ford HL. Epithelial-Mesenchymal Transition in Cancer: Parallels Between Normal Development and Tumor Progression. J Mammary Gland Biol Neoplasia 2010; 15: 117–134.

28. Zhen Z, Guo X, Liao R, Yang K, Ye L, You Z. Involvement of IL-10 and TGF-β in HLA-E-mediated neuroblastoma migration and invasion. Oncotarget 2016; 7: 44340–44349.

29. Shi Q, Wu H, Li Y, et al. Inhibition of Wilms' Tumor Proliferation and Invasion by Blocking TGF-β Receptor I in the TGF-β/Smad Signaling Pathway. BioMed Research International 2020; 2020: 1–10.

30. Aref D, Moffatt CJ, Agnihotri S et al. Canonical TGF-β Pathway Activity Is a Predictor of SHH-Driven Medulloblastoma Survival and Delineates Putative Precursors in Cerebellar Development: TGF-β Activity Is Prognostic in Medulloblastoma. Brain Pathology 2013; 23: 178–191.

31. Taran K, Frączek T, Sikora-Szubert A et al. The first investigation of Wilms' tumour atomic structure-nitrogen and carbon isotopic composition as a novel biomarker for the most individual approach in cancer disease. Oncotarget 2016; 7: 76726–76734.