ЕКСПЕРИМЕНТАЛЬНО-ТЕОРЕТИЧНИЙ

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THE NEW APPROACH FOR STUDY THE GROWTH REGULATION OF VARIOUS FORMS OF CHILDREN HEMANGIOMA

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It is known that disorders the expression of growth factors may trigger pathological processes in the body. For example, there is an assumption that the disturbance of positive and negative regulation of growth factors (VEGF and bFGF) can be associated with the development of hemangioma. Nowadays the subject of intense study is to investigate the expression of protein factors presence of which plays a leading role in angiogenesis at various stages of formation of hemangiomas. The partial examination of molecular factors controlling the growth and the evolution revealed that the identification of various factors is phase specific. Tissue inhibitor metalloproteinase 1, which is known as the inhibitor of a new blood vessels formation, is identified only in the involution phase. IFN also described as a potential endogenous inhibitor of hemangiomas proliferation. This research describes the biological characteristics of hemangioma in the various phases of its formation and identifies the possible signaling ways of the growth and the involution of hemangiomas, however, a more accurate description of them is still needed [1, 2].

It has been previously shown that endogenous thermostable protein complex isolated from the pro- and various organs of eukaryotic animals regulates cell proliferation of homologous tissue. The endogenous protein complex does not show species specificity, but reveals tissue-specificity with respect to terminally differentiated cells. It is shown that the active component of the thermostable protein complex (TPC) with low molecular weight (12-17 kD) decreases the proliferation of the homological cells through inhibition of RNA synthesis. Therefore it was interesting to determine the cell proliferation of different degree of transformation that regulated by endogenous thermostable protein complex. It is established, that the component of TPC with low molecular weight is not identified in the human kidney cancer cells [3,4]. Therefore it was interesting to determine the cell proliferation regulating endogenous thermostable protein in cells with different degree of transformation.

The aim of our study was the identification of the thermostable protein complexes in the cells of infant's hemangiomas.

Material and methods

The sources used in the experiments were postoperative material from Infants hemangiomas (capillary) received from Tbilisi State Medical University G. Zhvania Academic Clinic of Pediatry, and brain and pancreatic tissues from infant and adult rats. The influence of protein complex from capillary hemangioma was studied in infant (10-12 g) white rats.

Animals were divided into two groups: I - control (intact animals); II – test group. The mitotic index was determined for estimation of proliferative activity.

The protein complex (200γ) were injected in test group animals intraperitoneally. After an hour, colchicine was injected in both groups, the material (brain, pancreas) was taken after 2 hours from colchicine injection. The tissues were fixed in the 4% paraformaldehyde solution prepared in 0.1M phosphate buffered saline pH7.4. The samples were embedded in paraffin, then sectioned using a microtome and stained with H&E. Tissue samples were studied under a light microscope.

The thermostable protein complexes were obtained by alcohol extraction from the tissue of hemangioma and from adult rat pancreas as described [5]. The tissue was rinsed with the physiological solution and crushed. Aqueous homogenates were prepared in a tissue/distilled cold water ratio of 1:8. The homogenates were saturated step-wise with 96% ethanol to obtain the 81% ethanol fraction, which was boiled in a water bath (100^0C) for 20 min, cooled and centrifuged (600g, 15 min). The supernatant was frozen in liquid nitrogen and dried in an absorptive-condensate lyophilizer. As a result, a residue of the

protein complex white powder soluble in water was obtained. The samples were kept at 4^0C.

Gel electrophoresis assay was used for the comparative analysis of the complex components. Gel electrophoresis assay was carried out by method of Davis [6]. The polyacrylamide gel with concentration gradient of 10- 25% was used. The protein samples were diluted in buffer (0.5 M Tris HCl pH-6.8; 50% glycerol 0.05% bromophenol blue) and were separated electrophoretically (power - 14 mA, voltage - 100V). Gel was stained with silver nitrate.

Results and discussion.

We have previously studied the impact of TPC obtained from cavernous hemangioma on prolipherative activity of the heterotypic intact cells (adult rat pancreas and brain cells). Investigations have revealed that the TPC from cavernous hemangioma does not have the ability to inhibit the cell proliferation of the heterotypic tissue. In particular, the mitotic index of pancreas and brain cell of the infant rat from the experimental groups does not change as compared to the control. Also there was not revealed a substantial difference in the quantitative content of the protein components.

In the present work we obtained the TPC from children capillary hemangioma tissues and characterized by comparative analysis. Figure 1 presents the electrophoretic analysis of the TPC from the children's capillary hemangioma. In order to identify the TPC components the TPC from adult rat pancreas as a control were used (Figure 1). As shown in Figure, in compare to control sample the TPC from the children capillary hemangioma is different in the quantitative content, as its low molecular weight (12-17 KD), as well as a relatively high-molecular component. The most important difference is observed in the active component of TPC (low molecular weight). As mentioned above, such a difference of the thermostable protein complex from cavernous hemangiomas was not identified.

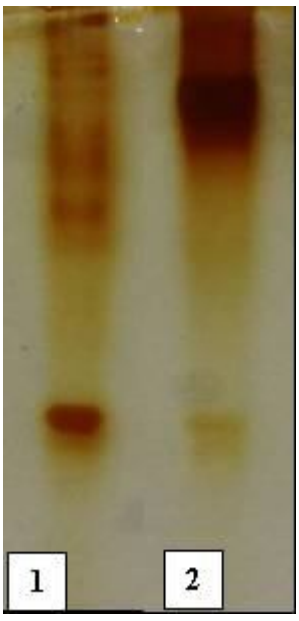

Figure 1. Gel electrophoresis of the TPC obtained from adult rat pancreas (1) and children capillary hemangioma (2).

The effect of the TPC from Children capillary hemangioma on the proliferative activity of the brain cells of the infant rats also were studied. It has been determined that the intraperitoneal injection of mentioned TPC decreases the mitotic activity of heterotypic cells (rat brain). In particular, the colchicine mitotic index is reduced approximately by 50 percent compared to the control (Figure 2).

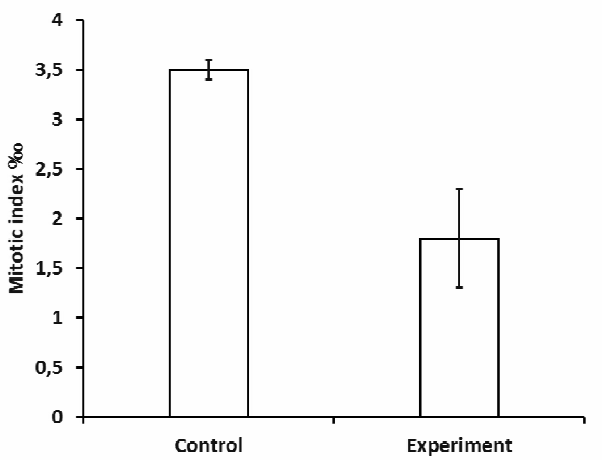

Figure 2. Influence of TPC from capillary hemangioma on the mitotic activity of the infant rat brain cells (p<0,01).

Conclusion.

On the bases of our results we can conclude that the thermostable protein complex is different in various forms of infant hemangiomas. The results also imply that the children capillary hemangiomas cells contain the thermostable protein complex, which has ability to inhibit the growth of the heterotypic cells of the infant organisms.

References:

1. G.V. Sherbet. Growth Factors and Their Receptors in Cell Differentiation, Cancer and Cancer Therapy. // Elsevier, 2011.- 347 pp.
2. Odile Enjolras, Michel Wassef and Rene Chapot. Introduction: ISSVA Classification. // Color Atlas of Vascular Tumors and Vascular Malformations (Cambridge University Press), 2014.-11pp.
3. Dzidziguri D., Aslamazishvili T., Chkhobadze M., Khorava P., Chigogidze T., Managadze L. The influence of white rat protein factor on transcriptional activity of normal and transformed cells //Proc. Georgian Acad. Sci., Biol. Ser. B. ,2004. - 2(1-2). – P. 30-35.
4. Rukhadze M.D., D.V.Dzidziguri, N.M.Giorgobiani and S.M.Kerkenjia. The study of growth inhibitive protein factor by various mode of HPLC and estimation of its binding with drugs Biomedical Chromatography// 2005. -N19. – P. 36-42.
5. I.Modebadze, M. Rukhadze, E. Bakuradze, D. Dzidziguri. Pancreatic Cell Proteome - Qualitative Characterization And Function// Georgian Medical News, 2013.-№ 7-8-P.71-77.

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Резюме

Целью нашего исследования было определение термостабильных белковых комплексов в клетках гемангиом детей.

Для определения термостабильных белковых комплексов из компонентов капиллярных гемангиом детей были использованы ТБК из взрослой поджелудочной железы крыс как контроль. ТБК из капиллярной гемангиомы детей отличаются от ТБК контрольного образца в количественном содержании как низко (12-17 KD), так и высокомолекулярной фракции. Изучено также влияние ТБК из компонентов гемангиом детей на пролиферативную активность клеток мозга новорожденных крыс. Было установлено, что внутрибрюшинное введение упомянутого ТБК снижает митотическую активность гетеротипических клеток мозга крыс.

Ключевые слова: капиллярная гемангиома у детей, термостабильный протеиновый комплекс.

Резюме

Метою нашого дослідження було визначення термостабільних білкових комплексів в клітинах гемангіом дітей.

Для визначення термостабільних білкових комплексів з компонентів капілярних гемангіом дітей були використані ТБК з дорослою підшлункової залози щурів як контроль. ТБК з капілярної гемангіоми дітей відрізняються від ТБК контрольного зразка в кількісному змісті як низько, так і високомолекулярної фракції. Вивчено також вплив ТБК з компонентів гемангіом дітей на активність клітин мозку новонароджених щурів. Було встановлено, що внутрішньочеревинне введення згаданого ТБК знижує мітотичну активність гетеротипічних клітин мозку щурів.

Ключові слова: капіляна гемангіома у дітей, термостабільний протеїновий комплекс.

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Summary

The aim of our study was the identification of the thermostable protein complexes in the cells of infant's hemangiomas.

In order to identify the TPC from children capillary hemangioma components the TPC from adult rat pancreas as a control were used. In compare to control sample the TPC from the children capillary hemangioma is different in the quantitative content, as its low molecular weight (12-17 KD), as well as a relatively high-molecular component. The effect of the TPC from Children capillary hemangioma on the proliferative activity of the brain cells of the infant rats also were studied. It has been determined that the intraperitoneal injection of mentioned TPC decreases the mitotic activity of heterotypic cells (rat brain).

Keywords: children capillary hemangioma, thermostable protein complex.