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The role of the transcription factor Sox2 and cytokeratins in the formation and development of the gastroesophageal junction epithelial cell differon

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The source of the origin of the epithelium of the cardiac part stomach mucosa has been repeatedly discussed in the literature and different variants of the transformation of the epithelium as manifestation of normal anatomical peculiarities of a man and as a result of changing the program of stem cell differentiation, migration of bone marrow cells, transdifferentiation of simple columnar epithelium have been proposed. Probably it is related to difficulties of studying insignificant in size epithelium of the cardiac mucosa itself and establishment of connection of the duodenogastroesophageal reflux with the development of metaplasia in the epithelium of the terminal department of the esophagus mucosa, which resembles its structure in the cardiac part of the stomach. The purpose of the research was to study the expression of the transcription factor Sox2 and the distribution of cytokeratins in the epithelium of the gastroesophageal zone during the stages of the embryonic and fetal periods of ontogenesis. According to the purpose of the research, an immunohistochemical analysis of the epithelial differon of the esophageal-gastric junction (GEJ) was used. The current study was carried out on 169 human embryos and fetuses of gestational age from 4-5 till 38 weeks. It was established that the transcription factor Sox2 is expressed in basal epitheliocytes of GEJ in all terms of observation and plays a major role in the development, differentiation and formation of the epithelial cell lineage of GEJ. The peculiarity of expression of cytokeratin 7 was positive marking in the cytoplasm of spinosum epitheliocytes, despite the negative expression in the basal layer. It showed weak expression in the epitheliocytes of the esophageal part of the GEJ in the embryonic period with an increased reaction in the embryo-fetal period and with subsequent disappearance, starting at 14 weeks in the early fetal period. For the cardiac mucous membrane GEJ was characterized by its moderate expression on all terms of observation. Cytokeratin 8/18 is embryo-fetal for the esophageal part of the esophagus, as it is defined in early periods of embryogenesis and disappears in the late period (28-38 weeks). For the cardiac mucous membrane GEJ was characterized by its moderate expression on all terms of observation. Cytokeratin 14, unlike CK7 and CK8/18, was localized in the cytoplasm and membranes of basal epitheliocytes of the esophageal part of the mucosa from the 17 gestational weeks and was absent in the gastrointestinal part of the GEJ throughout the prenatal period. Thus, our data on the expression of the transcription factor Sox2 and cytokeratins in the GEJ epithelial differon in the prenatal period of ontogenesis will improve the diagnostic accuracy in determining tissue or organ belonging and can be widely used in various GEJ diseases.

Keywords: gastroesophageal junction, immunohistochemical analysis, Sox2 transcription factor, cytokeratins, prenatal ontogenesis.

Introduction

One of the major transcription factors involved in the induction and maintenance of pluripotent stem cells is Sox2. As for the definition of stem cells in the upper gastrointestinal

tract, there are, at present, different, sometimes contradictory thoughts. Sox2 is proposed as one of the markers for the identification of stem cells in the esophagus and the

stomach, but its involvement in the differentiation and development of the gastroesophageal junction (GEJ) epithelial cell lineage remains poorly understood [6, 8, 13, 16].

Recognized immunohistochemical markers of the epithelium are cytokeratins - proteins of intermediate filaments (cytoskeleton) of epithelial cells. There are about 20 different cytokeratins that differ in amino acid composition, molecular weight, and isoelectric point. They are divided into two large groups: sour or type I (type A), which include cytokeratins 9-20 (CK9-CK20), and the main/neutral - 9 type II (type B), represented by CK1-CK8 [2, 9, 10, 17]. Cytokeratins are encoded by the genes of a large multigenic family (on chromosomes 11, 12 and 17). The different groups of epithelium are characterized by the corresponding groups of cytokeratins [3, 5].

In practical work for the immunocytochemical determination of the epithelial nature of atypical cells in the biopsies of tumors, antibodies against a specific cytokeratin or their wide spectrum (Pan-CK) are used. It is the cytokeratins responsible for the differentiation of the epithelium of various organs and the preservation of its cytoskeleton. Regarding the GEJ epithelial cell lineage, their distribution and role in the formation, ripening, cell migration and differentiation of the embryonic and epidermal epithelium of embryos and fetuses at present have not been sufficiently studied.

That is why the *purpose* of our study was to establish the expression of the transcription factor of Sox2 and the distribution of cytokeratins in the GEZ epithelium in the stages of embryonic and fetal periods of ontogenesis.

Materials and methods

The study was performed on embryos and fetuses of a person aged 4-5 up to 38 weeks of fetal development that developed in the uterus in the absence of explicit external and internal factors obtained from medical abortions or stillborn in relatively healthy women in the Vinnitsa Regional Anatomic Pathology Department (VRAPD) and maternity homes in the city of Vinnytsia and who died from causes not associated with GIT diseases.

Fetal preparations weighing more than 500.0 g were studied directly in VRAPD of Vinnitsa (Order of the Ministry of Health of Ukraine "On Approval of Instructions for Determining the Perinatal Period, Live Birth and Deadly Births, Procedure for Registration of Abandoned and Deadly Births" No. 179 dated 29.03.2006). The age of subjects was determined according to tables by T. Sadler (2001) on the basis of measuring the crown-rump length [4, 18, 19].

The total number of embryos and fruits was 169, which were distributed by age and crown-rump length (CRL) to the following groups (Table 1).

The objects under investigation were also distributed by age periodization by R.K. Danilov, T.G. Borova (2003), A.V. Mazurin, I.M. Vorontsov (2009), and included the following periods of prenatal development of a person [1, 4, 12, 21,

Table 1. Distribution of human embryos and fetuses according to age groups (M±m).

Age (weeks)	Quantity	CRL (mm)
4-5	6	6.100±0.300
6-7	6	15.10±0.60
8-9	7	19.40±0.50
10-11	10	39.02±0.50
12-13	11	58.72±2.27
14-15	12	93.11±5.10
16-17	15	122.2±2.7
18-20	19	152.9±3.5
21-24	20	192.1±1.8
25-28	13	230.9±3.1
29-32	14	264.8±1.7
33-36	20	302.9±1.4
37-38	16	341.5±5.7
Total	169	-

23]: embryonic period - from 5 to 6 weeks (35-42 days); embryo-fetal period - from 7 to 9 weeks (43-62 days); early fetal period - from 10 to 28 weeks (63-196 days); late fetal period - from 28 to 38 weeks (197-266 days by R.K. Danilov, T.G. Borova, 2003), [4].

The research has been carried out in accordance with the methodological recommendations "Compliance with ethical and legal norms and requirements in the course of scientific morphological research" and "Procedure for the seizure and use of biological objects from dead persons whose bodies are subject to forensic examination and pathological anatomical investigation for scientific purposes" [10, 13]. To determine morphological changes, general-histological (staining with hematoxylin-eosin) and immunohistochemical methods were used. For quantitative evaluation of the Sox2 transcription factor and expression of cytokeratins, a semi-quantitative method was used, according to which 4 categories were distinguished: 0 (-) - negative reaction (coloration <5% of cells), 1 (+) - weak coloration (positively colored 10-30% of cells), 2 (++) is a moderately pronounced reaction (most of the positively colored cells - 31-60%) and 3 (+++) - intense coloration (> 60% cells or almost all cells of the epithelium are positively colored). The coefficient of expression (CE) was calculated for each observation by the formula: $KE = \sum (i \times v) / 100$, where i - the intensity of the color in points (from 0 to 3), v - the percentage of stained cells (from 0 to 100% of the most a pronounced reaction in 10 fields of view at x400) for each value of "i". Immunohistochemical research was carried out on the basis of the Vinnitsa Regional Anatomic Pathology Department.

Microscopy and photographing of histological preparations were performed using an optical microscope OLIMPUS BX 41 with magnifications of 40, 100, 200 and 400 times. Receive and process photos, perform

morphometry and statistical processing using the "Quick PHOTO MICRO 2.3" program. When performing morphometric studies were guided by the basic principles laid out in the G.G. Avtandilova (2002) guide [2]. The obtained digital results were processed by a variational statistical method using Student tables.

Results

At the immunohistochemical analysis of GEJ epithelial cell lineage, the Sox2 transcription factor already showed strong expression in the nuclei of epitheliocytes both in the gastric epithelium (Fig. 1) and in the esophageal part of the GEJ (Fig. 2) at the embryonic period of the study. The intensity of the expression of this marker is presented in Table 2.

Upon further study of this marker, we observed its positive conjugation with mainly basal nuclei of epitheliocytes, both on embryo-fetal, and in early fetal periods in both parts. The late fetal period was characterized by a positive strong, sometimes moderate, expression of the transcription factor of Sox2 in the nuclei of basal epitheliocytes, despite its

Fig. 1. A strong expression of Sox2 in the nuclei of the epithelial cells of GM is indicated by an arrow. Stomach of embryo 4-5 weeks with CRL 6.1±0.3 mm. Immunohistochemical labeling with Sox2, x400.

Fig. 2. The Sox2 strong expression in the epithelial cells of the esophageal part of the GEJ is marked with an arrow. Esophagus of embryo 6-7 weeks with CRL 15.1±0.6 mm. Immunohistochemical labeling. Sox2, x400.

Table 2. Intensity of transcription factors Sox2 expression in cytoplasm of epitheliocytes of GEJ (M±m).

Age (weeks)	Sox2	
	Esophagus	Stomach
4-5	2.544±0.163	0.807±0.043
6-7	2.696±0.089	0.727±0.060
8-9	2.763±0.081	0.754±0.047
10-11	2.817±0.054	0.721±0.076
12-13	2.842±0.637	0.739±0.037
14-15	2.805±0.075	0.171±0.039
16-17	2.817±0.064	0.160±0.027
18-20	0.784±0.063	0.169±0.025
21-24	0.779±0.044	0.221±0.171
25-28	0.810±0.053	0.401±0.229
29-32	0.795±0.044	0.401±0.229
33-36	0.780±0.044	0.293±0.176
37-38	0.814±0.073	0.336±0.209

weak marking in the nuclei of surface epitheliocytes of both the gastrointestinal and esophageal parts of GEJ. The most potent expression in the immunohistochemical analysis of the transcription factor of Sox2 was observed in basal epitheliocytes of the gastric part of GEJ in the late fetal period, despite its weak expression, and sometimes the lack of marking in surface epitheliocytes.

The cytoplasmic marker of epithelial differentiation of high molecular weight cytokeratin 7 (CK7) during the embryonic period of the observation showed poor expression predominantly in the apical parts of the epithelial cell lineage of the esophagus and the stomach (Fig. 3). It intensively (+++) intensified during the formation of gastric pits, coinciding with the data of the next study [1, 21, 28], also in the gastric part of GEJ and at the beginning of the early fetal period in the esophageal part of the GEJ (Table 3).

The feature of its marking was the positive coloration of

Fig. 3. Weak expression of CK7 in the apical part of the esophagus epithelium cytoplasm is marked with an arrow. Esophagus of embryo 4-5 weeks with CRL 6.1±0.3 mm. Immunohistochemical labeling with CK7. x200.

Table 3. Intensity of expression of cytokeratin 7 in cytoplasm of epitheliocytes of GEJ (M±m).

Age (weeks)	Cytokeratin 7	
	Esophagus	Stomach
4-5	0.230±0.080	0.149±0.027
6-7	0.264±0.081	0.232±0.148
8-9	0.885±0.200	0.334±0.168
10-11	2.549±0.170	0.345±0.168
12-13	0.252±0.173	0.736±0.099
14-15	-	0.794±0.006
16-17	-	0.753±0.116
18-20	-	0.781±0.098
21-24	-	0.817±0.106
25-28	-	0.809±0.132
29-32	-	0.810±0.065
33-36	-	0.848±0.065
37-38	-	0.821±0.622

Fig. 4. Positive strong expression of CK8/18 in basal (arrow 1) and apical (arrow 2) cytoplasm epithelial cells and basal membrane of the esophagus. Esophagus of embryo 4-5 weeks with CRL 6.1±0.3 mm. Immunohistochemical labeling with CK8/18, x200.

this cytokeratin in undifferentiated epitheliocytes of gastric mucosa. Subsequently, the marking of CK7 increased from moderate at 9 week to a strong coloring at 11 week, which coincided with the acquisition of the properties of ciliated pseudostratified columnar epithelium. Cytokeratin 7 disappears already at the stratified squamous epithelium stage, cytokeratin 8 is enhanced in the stomach, and completely disappears in the esophageal epithelium at 37 week.

Immunohistochemical analysis of the cytokeratin 8 (CK8/18) in the GEJ epithelial cell lineage in the embryonic period (5-7 weeks) has established its moderate, sometimes vigorous expression, both in the apical and basal parts of the esophagus and stomach cytoplasm epitheliocytes. It should be noted that along with positive marking of cytoplasm, quite characteristic was fairly typical and strong expression of the marker in the basal membrane, which is stained in brown and well observed (Fig. 4).

Cytokeratin 8/18 was detected in the cytoplasm and plasmalemma of spinosum epitheliocytes of GEJ with perinuclear region localization of granules. In most cells, they were evenly distributed. Cytokeratins 8/18 coexpression observed in both esophageal and gastric epithelium of GEJ, while marking was intense in the early period of embryogenesis and insignificant at later dates (tab. 4).

Cytokeratin 8/18 is registered in plasmalemma in the esophagus up to 21 weeks and disappears from the 24 gestational weeks. At the end of the late fetal period, the epithelium of the esophageal part of the GEJ is negative in the esophagus, but with the appearance of a stratified squamous epithelium, it appears cytokeratin14 in basal epitheliocytes, which coincides with the subsequent study [15, 25, 26].

In the gastric part of the GEJ epithelial cell lineage, the expression of CK8/18 was moderate with a diffuse

Table 4. Intensity of expression of cytokeratin 8/18 in cytoplasm of epitheliocytes of GEJ (M±m).

Age (weeks)	Cytokeratin 8/18	
	Esophagus	Stomach
4-5	2.480±0.159	2.484±0.162
6-7	0.418±0.245	2.516±0.117
8-9	0.267±0.126	2.681±0.069
10-11	2.490±0.088	2.530±0.117
12-13	0.816±0.180	0.721±0.176
14-15	0.774±0.112	0.756±0.071
16-17	0.790±0.073	0.707±0.178
18-20	0.774±0.042	0.682±0.122
21-24	0.714±0.054	0.837±0.070
25-28	-	0.763±0.055
29-32	-	0.847±0.091
33-36	-	0.817±0.071
37-38	-	0.815±0.073

Fig. 5. Positive weak perinuclear expression of CK8/18 in the basal parts of the surface epithelium and glandular exocrinocytes is indicated by an arrow. Fetal stomach, 37-38 weeks with CRL 341.5±5.7 mm. Immunohistochemical labeling with CK8/18. x200.

distribution pattern of cytokeratins in the cytoplasm of epitheliocytes throughout the entire observation period. Marking prevailed in the gastric mucosa, where non-differentiated gastric epitheliocytes were localized (Fig. 5).

At immunohistochemical analysis of expression of cytokeratin 14 (CK14) it was found that it exhibited strong conjugation with basal epitheliocytes in the esophageal part of MM GEJ in the fetal period. From the 17th week, its appearance in the cytoplasm and plasmalemma basal epitheliocytes of the esophageal unit GEJ was recorded despite weak, sometimes moderate markings in the plasmalemma of suprabasal and superficial epitheliocytes. In this case, the positive marking remained in the data structural elements of MM GEJ throughout the fetal period to 38 weeks. The expression of this marker was absent in both the esophagus and the gastrointestinal part of the GEJ in the embryonic, embryo-fetal and early fetal periods. Also, its expression was negative in the epithelium of cardia part gastric mucosa in the late fetal period. It should be noted that in the fetal period, its expression in the cytoplasm coincided with the appearance in nuclei transcription factor p63 in the basal epitheliocytes of the esophageal part of the GEJ.

Thus, the data we obtained from the immunohistochemical analysis of GEJ epithelial cell lineage allow us to estimate the biological potential of cells, to establish tissue and organ affinity, and can be widely used in the diagnosis of non-tumor nature and tumors of the gastrointestinal tract to facilitate the accurate classification of tumors and to obtain significant prognostic information when use of new molecular targeting therapy [22, 23, 24].

Discussion

The study of patterns of the implementation of mechanisms of histogenesis (a set of processes that lead to the formation and restoration of tissues in the course of individual development) is now impossible without determining the role of these processes of stem and progenitor cells, the peculiarities of the development and interaction of different cellular clones in the early stages of histogenesis [7, 17, 22]. Intensive study of stem cells significantly increased the level of our knowledge about the molecular genetic mechanisms of histogenesis and the development of methods for their regulation. Stem cells are a hierarchy of special undifferentiated pluripotent cells of living organisms that can asymmetrically divide with the formation of a similar parent (self-reproduction) and a new (progenitor) cell that is differentiated into a certain type of cell. Progenitor cells, in contrast to pluripotent stem cells, have stable biomarkers to distinguish between them and their offspring from cells of other cell lineages, the ability to proliferate is much lower than that of pluripotent stem cells. As for the epithelial cell lineage of both the esophagus and GEJ, the issue of identification of the stem cell precursor remains open today. Thus, their role is mainly offered by basal epitheliocytes of the epithelial cell lineage, but various authors find cells with various molecular genetic markers.

Thus, basal epitheliocytes with positive expression of the transcription factor p63 were proposed by some researchers [13, 17], other authors - CD34 [17, 21], as well as cells with positive expression of the transcription factor of Sox2 [22]. In our opinion, this is due to the fact that the stem cell precursor of the epithelial cell lineage, due to heterogeneity, that is, the expression of several different transcription factors, can be differentiated in different directions. Further switching or shutdown of a particular transcription factor under the influence of factors not yet known to date leads to a change in the cellular phenotype. Thus, in our previous study, we recorded the disappearance of the expression of the transcription factor p63 at the end of the embryonic stage of embryogenesis (7 weeks), resulting in the appearance of a new epithelial cell lineage in the mucosa membrane of GEJ, in the esophagus part of the ciliated pseudostratified columnar epithelium, and in the cardia part - cuboidal and over time, the columnar epithelium, which remained negative for marking p63 until the end of the study. In the esophagus part with the appearance of a stratified squamous epithelium, the transcription factor p63 reappeared in basal epitheliocytes of stratified squamous epithelium [7, 16].

Unlike p63, the transcription factor of Sox2 was observed at all terms of the observation, which was detected predominantly in the nuclei of basal epitheliocytes of GEJ, consistent with the results of subsequent studies [25, 26, 27]. It should be noted that GEJ epithelial cell lineage cells had a strong positive expression for several molecular genetic markers, such as p63, Sox2, CK14, CK8/18, weak for CK7 for 4-6 weeks of the embryonic period. This confirms the presence in this epithelial cell lineage of pluripotency of the properties of stem cells. Over time, from 7-8 gestational weeks, these cells lost the ability to express one or another molecular genetic marker, indicating their transdifferentiation to the progenitor cell (stem cell precursor) of GEJ epithelial cell lineage.

The weak expression of CK7 in the apical sections of the esophagus epitheliocytes of the esophagus was observed by us from 5 week, which does not coincide with the data of the subsequent studies that recorded its appearance from 12 week of gestation with respect to cytokeratin 8/18, then its labeling is consistent with the results of foreign studies that also noted its expression at the early embryonic period. In the epithelial cell lineage of the gastric part of GEJ, the expression of CK8/18 was moderate and, unlike the esophagus, remained throughout the study period.

According to our data, cardia part of gastric mucosa is congenital rather than the result of meta-plastic transformations that coincided with the work of foreign authors [8, 16, 21, 26, 27]. From 5 to 7 weeks, the epithelial cell lineage remains heterogeneous with the properties of both the esophagus, gastric and intestinal phenotypes, differentiation begins with 8 weeks, determination and specialization in stratified squamous epithelium in the stomach from 10 weeks. Thus, based on our data on expression of transcription factors and cytokeratins, the GEJ

epithelial cell lineage can distinguish pluripotent stem cells with heterogeneous properties present at 4-6 weeks and have positive expression for p63, Sox2, CK7, CK8/18, and CK14, indicating their ability to differentiate into various cellular phenotypes.

Based on the data obtained in this and previous studies, the stem cell precursor stratified squamous epithelium in our opinion is those that had positive expression of p63, CK14 but lost the ability to express CK7, CK8/18 for stratified squamous epithelium. As for the columnar epithelium cardia part of GEJ, for its stem cell precursor, the positive expression of CK7 and CK8/18 was characteristic on all observation periods, with the advantage of marking CK8/18 and negative for p63 starting from 7-8 gestational weeks, despite weak expression Sox2 on all terms of the study.

The results of our study may be useful in developing methods for purposeful correction of key mechanisms of histo-, morpho-, organogenesis, as well as the formation of a new direction in science - regenerative biology and medicine. The most promising direction for this is the use of genetic, cellular, tissue and organ biotechnologies for the treatment of various diseases, as well as the cloning of cells and animals [15, 20].

Thus, the data we obtained from the immuno-histochemical analysis of GEJ epithelial cell lineage allow us to estimate the biological potential of cells, to establish tissue and organ affinity, and can be widely used in the diagnosis of non-tumor nature and tumors of the gastrointestinal tract to facilitate the accurate classification of tumors and to obtain significant prognostic information when use of new molecular targeting therapy [12, 23, 24].

Further studies and the establishment of the patterns of expression of the transcription factor of Sox2 and human cytokeratins in the GEJ epithelial cell lineage in the prenatal period of ontogenesis will complement current data on its morphology and increase the accuracy of the diagnosis and evaluation of pathological changes in the GEJ mucosa in various GEJ diseases.

Conclusions

1. The expression of the transcription factor of Sox2 was observed predominantly in basal epitheliocytes of GEJ

References

- [1] Ahmed, Y. A., El-Hafez, A. A. E., & Zayed, A. E. (2016). *Histological and Histochemical Studies on the Esophagus, Stomach and Small Intestines of Varanus niloticus*. arXiv preprint arXiv: 1610.01717.
- [2] Avtandilov, G. G. (2007). *Basics of pathoanatomical practice. Manual (third edition amended)*. M.: Russian Medical Academy of Postgraduate Education.
- [3] Chen, X., Qin, R., Liu, B., Ma, Y., Su, Y., Yang, C. S., ... & Shaheen, N. J. (2008). Multilayered epithelium in a rat model and human Barrett's esophagus: similar expression patterns of transcription factors and differentiation markers. *BMC gastroenterology*, 8(1), 1. doi: 10.1186/1471-230X-8-1.
- [4] Danilov, R. K. & Borovaya, T. G. (2003). *General and medical embryology*. SPb.: SpecLit. ISBN: 5-299-00208-4
- [5] De Hertogh, G., Van Eyken, P., Ectors, N., & Geboes, K. (2005). On the origin of cardiac mucosa: A histological and immunohistochemical study of cytokeratin expression patterns in the developing esophagogastric junction region and stomach. *World journal of gastroenterology: WJG*, 11(29), 4490. doi: 10.3748/wjg.v11.i29.4490.
- [6] DeWard, A. D., Cramer, J., & Lagasse, E. (2014). Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell reports*, 9(2), 701-711.
- [7] Gunther, C., Neumann, H., & Vieth, M. (2014). Esophageal epithelial resistance. *Dig Dis*, 32, 6-10.
- [8] Jacobs, I. J., Ku, W. Y., & Que, J. (2012). Genetic and cellular mechanisms regulating anterior foregut and esophageal

and in all terms of observation, which may indicate its central role in the development, differentiation and formation of the GEJ epithelial cell lineage. The transcription factor p63 is involved in the formation of stratified squamous epithelium, its moderate expression was characteristic of the undifferentiated epithelium in the embryonic period and the already differentiated epithelium of the late fetal period. Its disappearance of the "exception" at the 8th gestational week in cardia mucous membrane of GEJ indicates transdifferentiation, a change in the cellular phenotype on the columnar epithelium.

2. The distribution of cytokeratins was characterized by heterogeneity, with the expression of cytokeratin 7 being positive in the cytoplasm of spinosum epitheliocytes, despite the negative expression in the basal layer. It showed weak expression in epitheliocytes of the esophageal part of the GEJ in the embryonic period with an increased reaction in the embryo-fetal period and subsequently disappeared from the 14th week in the early fetal period. For cardia mucous membrane of GEJ was characterized its moderate expression on all terms of observation

3. Cytokeratin 8/18 is an embryo-fetal for the esophagus part of the esophagus, as it was determined in the early periods of embryogenesis and disappears in the late period (28-38 weeks). Cardia of the mucous membrane of GEJ was characterized by its moderate expression on all terms of observation.

4. Cytokeratin 14, in contrast to CK7 and CK8/18, has been localized in the cytoplasm and membranes of basal epitheliocytes of the esophageal part of the GEJ mucosa membrane from the 17th gestational week and was absent in the gastric part of the GEJ throughout the prenatal period.

5. Based on the data of immunohistochemical analysis, it is possible to separate the major GEJ epithelial cell lineage in the prenatal period of ontogenesis: 4-6 gestational weeks - pluripotent stem cells with positive expression of p63, Sox2, CK8/18, CK7; 7-38 weeks - progenitor cell stratified squamous epithelium of the esophageal part of GEJ with positive expression of Sox2, p63, CK14; 14-38 weeks - progenitor cell of columnar epithelium cardia part of GEJ with positive expression of Sox2, CK8/18, CK7.

- development. *Developmental biology*, 369(1), 54-64.
- [9] Jazii, F. R. (ed.) (2012). Esophageal Cancer: Cell and Molecular Biology, Biomarkers, Nutrition and Treatment. *Intech. P*. ISBN: 978-953-51-0223-6
- [10] Kalabis, J., Oyama, K., Okawa, T., Nakagawa, H., Michaylira, C. Z., Stairs, D. B., ... & Rustgi, A. K. (2008). A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. *The Journal of clinical investigation*, 118(12), 3860-3869.
- [11] Kulinichenko, V. L., Mishalov, V. D., Tchaikovsky, Yu. B., Pustovit, S. V., & Voichenko, V. V. (2007). Compliance with ethical and legislative norms and requirements in the course of scientific morphological research. *Morphology*, 1(4), 134-135.
- [12] Mazurin, A. B. & Vorontsov, A. B. (2009). *Propedeutics of childhood diseases*. Foliant, Russia. ISBN: 978-5-93929-184-2
- [13] McKeon, F. (2004). p63 and the epithelial stem cell: more than status quo? *Genes & development*, 18(5), 465-469.
- [14] Mishalov, V. D., Voychenko, V. V., Malysheva, T. A., Dibrova, V. A., Kuzik, P. V., & Yurchenko, V. T. (2018). The procedure for the extraction and use of biological objects from dead persons whose bodies are subject to forensic examination and pathologoanatomical research, for scientific purposes. *Special edition of the newspaper "Osvita Ukrainy"*, 3-13.
- [15] Namiot, Z., Sarosiek, J., Marcinkiewicz, M., Meade, C., & Edmunds Richard, W. (1994). McCallum Declined human esophageal mucin secretion in patients with severe reflux esophagiti. *Digestive Diseases and Sciences December*, 39(12), 2523-2529.
- [16] Özeğ, ve Mide Gelişimi. (2017). Development of the esophagus and stomach. *Bezmialem Science*, 5, 175-82. DOI: 10.14235/bs.2017.811.
- [17] Rishniw, M., Rodriguez, P., Que, J., Burke, Z. D., Tosh, D., Chen, H., & Chen, X. (2011). Molecular aspects of esophageal development. *Annals of the New York Academy of Sciences*, 1232(1), 309-315. doi: 10.1111/j.1749-6632.2011.06071.x.
- [18] Sapin, M. R., Nikolenko, V. N., Chava, S. V., Alekseeva, T. N., & Nikityuk, D. B. (2013). Questions classification and morphogenesis of the glands of the walls of hollow internal organs. *Journal of Anatomy and Histopathology*, 1, 9-17.
- [19] Sadler, T. V. (2001). *Medical Embryology by Langman* (textbook). Translation from English Edited by O.D. Lutsyk. Lviv: Nautilus. ISBN: 966-95745-3-6.
- [20] Seery, J. P. (2002). Stem cells of the oesophageal epithelium. *Journal of cell science*, 115, 1783-1789.
- [21] Ventura, A., do Nascimento, A. A., dos Santos, M. A. J., Vieira-Lopes, D. A., Sales, A., & Pinheiro, N. L. (2013). Histological Description of Morphogenesis of the Gastroesophageal Mucosa of Gallus gallus Domesticus (Linnaeus, 1758). *International Journal of Morphology*, 31(4), 1331-1339. DOI: 10.4067/S0717-95022013000400030
- [22] Wang, D. H., Tiwari, A., Kim, M. E., Clemons, N. J., Regmi, N. L., Hodges, W. A., ... & Zhang, Q. (2014). Hedgehog signaling regulates FOXA2 in esophageal embryogenesis and Barrett's metaplasia. *The Journal of clinical investigation*, 124(9), 3767-3780. doi: 10.1172/JCI66603.
- [23] Wang, X., Ouyang, H., Yamamoto, Y., Kumar, P. A., Wei, T. S., Dagher, R., ... & Crum, C. P. (2011). Residual embryonic cells as precursors of a Barrett's-like metaplasia. *Cell*, 145(7), 1023-1035. doi: 10.1016/j.
- [24] Weaver, J. M., Ross-Innes, C. S., Shannon, N., Lynch, A. G., Forshew, T., Barbera, M. ... Fitzgerald, R. C. (2014). Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. *Nature genetics*, 46(8), 837-843. doi: 10.1038/ng.3013
- [25] Willet, S. G., & Mills, J. C. (2016). Stomach organ and cell lineage differentiation: from embryogenesis to adult homeostasis. *Cellular and molecular gastroenterology and hepatology*, 2(5), 546-559. DOI: 10.1016/j.jcmgh.2016.05.006
- [26] Zhang, Y., Jiang, M., Kim, E., Lin, S., Liu, K., Lan, X., & Que, J. (2017). Development and stem cells of the esophagus. In: *Seminars in cell & developmental biology*, 66, 25-35. Academic Press. doi: 10.1016/j.semcdb.2016.12.008
- [27] Zhou, H., Greco, M. A., Daum, F., & Kahn, E. (2001). Origin of cardiac mucosa: ontogenic consideration. *Pediatric and developmental pathology*, 4(4), 358-363.

РОЛЬ ТРАНСКРИПЦІЙНОГО ФАКТОРА SOX2 ТА ЦИТОКЕРАТИНІВ У ФОРМУВАННІ ТА СТАНОВЛЕННІ ЕПІТЕЛІАЛЬНОГО ДИФЕРОНУ ГАСТРОЕЗОФАГЕАЛЬНОГО З'ЄДНАННЯ

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В літературі неодноразово обговорювали питання щодо джерела походження епітелію слизової оболонки кардіальної частини шлунка та пропонували різні варіанти трансформації епітелію як прояву нормальної анатомічної особливості людини, так і внаслідок зміни програми диференціювання стовбурової клітини, міграції клітин кісткового мозку, трансдиференціації одношарового циліндричного епітелію. Ймовірно, це пов'язано з труднощами вивчення незначного за розмірами епітелію слизової оболонки власне кардії й встановленням зв'язку дуоденогастроєзофагеального рефлексу з розвитком метоплазії в епітелії слизової оболонки термінального відділу стравоходу, котрий нагадує його будову в кардіальному відділі шлунку. Мета дослідження - вивчити експресію транскрипційного фактора Sox2 та розподіл цитокератинів в епітелії гастроєзофагеальної зони (ГЕЗ) на етапах ембріонального та фетального періодів онтогенезу. Згідно з метою дослідження використали імуногістохімічний аналіз епітеліального диферону стравохідно-шлункового переходу (СШП). Дослідження виконано на 169 ембріонах та плодах людини віком від 4-5 до 38 тижнів внутрішньоутробного розвитку. Встановлено, що транскрипційний фактор Sox2 експресується в базальних епітеліоцитах ГЕЗ на всіх термінах спостереження та виконує головну роль у розвитку, диференціації та становленні епітеліального диферону ГЕЗ. Особливістю експресії цитокератину 7 було позитивне маркування у цитоплазмі остистих епітеліоцитів попри негативну експресію в базальному шарі. Він проявив слабку експресію в епітеліоцитах стравохідної частини ГЕЗ в ембріональному періоді з посиленням реакції у ембріофетальному періоді та з подальшим зникненням, починаючи з 14 тижня у ранньому фетальному періоді. Для кардіальної слизової оболонки ГЕЗ була характерна його помірна експресія на всіх строках спостереження. Цитокератин 8/18 є ембріофетальним для стравохідної частини стравоходу, так як визначається на ранніх періодах ембріогенезу та зникає у пізньому періоді (28-38 тижнів). Для кардіальної слизової оболонки ГЕЗ була характерна його помірна експресія на всіх строках спостереження. Цитокератин 14, на відміну від СК7 та СК8/18, локалізувався в цитоплазмі та мембранах базальних епітеліоцитів стравохідної частини слизової оболонки ГЕЗ починаючи з 17 гестаційного тижня та був відсутнім у шлунковій частині СШП упродовж всього пренатального періоду. Таким чином, отримані нами дані щодо експресії

транскрипційного фактора Sox2 та цитокератинів в епітеліальному дифероні ГЕЗ у пренатальному періоді онтогенезу дозволять підвищити точність діагностики при визначенні тканинної або органної належності та можуть широко застосовуватися при різних захворюваннях СШП.

Ключові слова: гастрозофагеальне з'єднання, імуногістохімічний аналіз, транскрипційний фактор Sox2, цитокератини, пренатальний онтогенез.

РОЛЬ ТРАНСКРИПЦИОННОГО ФАКТОРА SOX2 И ЦИТОКЕРАТИНОВ В ФОРМИРОВАНИИ И СТАНОВЛЕНИИ ЭПИТЕЛИАЛЬНОГО ДИФФЕРОНА ГАСТРОЗОФАГЕАЛЬНОГО СОЕДИНЕНИЯ

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В литературе неоднократно обсуждали вопрос относительно источника происхождения эпителия слизистой оболочки кардиальной части желудка и предлагали различные варианты трансформации эпителия как проявление нормальной анатомической особенности человека, так и вследствие изменения программы дифференцировки стволовой клетки, миграции клеток костного мозга, трансдифференциации однослойного цилиндрического эпителия. Вероятно, это связано с трудностями изучения незначительного по размерам эпителия слизистой оболочки собственно кардии и установлением связи дуоденогастрозофагеального рефлекса с развитием метаплазии в эпителии слизистой оболочки терминального отдела пищевода, который напоминает его строение в кардиальном отделе желудка. Цель исследования - изучить экспрессию транскрипционного фактора Sox2 и распределение цитокератинов в эпителии гастрозофагеальной зоны (ГЭЗ) на этапах эмбрионального и фетального периодов онтогенеза. Согласно с целью исследования использовали иммуногистохимический анализ эпителиального дифферона пищеводно-желудочного перехода (ПЖП). Исследование выполнено на 169 эмбрионах и плодах человека в возрасте от 4-5 до 38 недель внутриутробного развития. Установлено, что транскрипционный фактор Sox2 экспрессируется в базальных эпителиоцитах ГЭЗ на всех сроках наблюдения и выполняет главную роль в развитии, дифференциации и становлении эпителиальных дифферонов ГЭЗ. Особенностью экспрессии цитокератина 7 была положительная маркировка в цитоплазме остистых эпителиоцитов несмотря на негативную экспрессию в базальном слое. Он проявил слабую экспрессию в эпителиоцитах пищеводной части ГЭЗ в эмбриональном периоде с усилением реакции в эмбриофетальном периоде и с дальнейшим исчезновением, начиная с 14 недели в раннем фетальном периоде. Для кардиальной слизистой оболочки ГЭЗ была характерна его умеренная экспрессия во все сроки наблюдения. Цитокератин 8/18 является эмбриофетальной для пищеводной части ГЭЗ, так как определяется на ранних периодах эмбриогенеза и исчезает в позднем фетальном периоде (28-38 недель). Для кардиальной слизистой оболочки ГЭЗ была характерна его умеренная экспрессия на всех сроках наблюдения. Цитокератин 14, в отличие от CK7 и CK8/18, локализовался в цитоплазме и мембранах базальных эпителиоцитов пищеводной части слизистой оболочки ГЭЗ, начиная с 17 гестационной недели, и был отсутствующим в желудочной части ГЭЗ в течение всего пренатального периода. Таким образом, полученные нами данные экспрессии транскрипционного фактора Sox2 и цитокератинов в эпителиальном диффероне ГЭЗ в пренатальном периоде онтогенеза позволят повысить точность диагностики при определении тканевой или органной принадлежности и могут широко использоваться при различных заболеваниях ПЖП.

Ключевые слова: гастрозофагеальное соединение, иммуногистохимический анализ, транскрипционный фактор Sox2, цитокератини, пренатальный онтогенез.