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Comparative study of HER2, EGFR, p53 and PTEN expression in the human gastrointestinal tract during fetal period

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Abstract

Introduction: HER2, EGFR, p53 and PTEN are important in organization of the germ layers, in embryonic development and morphogenesis, in the development and differentiation of certain organ systems and in embryonic morphogenesis. Our goal is the comparative examination of the expression of these markers in the digestive tract of 9–24-week-old fetuses. *Materials and Methods*: We studied using immunohistochemical techniques esophagus, stomach, small and large intestine tissue samples collected from 18 post mortem fetuses of 9–24 weeks. *Results*: HER2 and PTEN expression appears as early as the 9–12 weeks period in the digestive tract, but HER2 expression decreases in the 21–24 weeks period and then disappears. EGFR expression appears only during the 13–16 weeks period. The expression of p53 is strong until week 21, and then it is restricted to the deeper layers of the epithelium. *Conclusions*: Our findings suggest that these markers have role also in the fetal period and complete the scarce data found in literature about the expression of the studied markers in the development of the digestive tract.

Keywords: HER2, EGFR, PTEN, p53, fetal development, gastrointestinal tract.

Introduction

The general histological structure of the digestive tract is already established in the embryonic period, but the development of certain layers ends in the fetal period. Development along the digestive tract occurs from rostral to caudal, from right to left and from the inside to outside.

Differentiation of the mucosa occurs in a similar manner in all segments of the digestive tract. Before post-fertilization week six, the simple endodermal epithelium, which appears along with the bilaminar embryonic disc, transforms into stratified columnar primordial epithelium. During the week eight, the thick epithelium differentiates into tall simple or pseudostratified columnar epithelium. Between weeks 8 and 10, the latter quickly differentiates into the epithelium specific to each segment of the digestive tract [1].

The EGFR (epidermal growth factor receptor) activation is important in embryonic development, tissue homeostasis and wound healing [2]. EGFR-deficient mice presented proliferation, migration and differentiation disorders in the skin, central nervous system, digestive tract, lung, liver and kidney tissue [3]. Mutations leading to increased EGFR activity and overexpression were demonstrated in several tumors [2, 4, 5].

Several studies upheld the role played by HER2 (human epidermal growth factor receptor-2) in the development of certain organ systems: heart; lungs; growth and differentiation of the epithelium [6–9].

The p53 protein has a role in embryonic morphogenesis [10, 11]; neuronal, osteogenic, myogenic, adipogenic differentiation and also in hematopoesis [12].

PTEN (phosphatase and tensin homologue) has role in embryogenesis, organization of the germ layers [13–15], the development of the lymphatic [16], skeletal [17, 18], and nervous systems, development of the thyroid gland, kidney [19], and also in neuronal differentiation [20].

The above-mentioned markers are studied in current studies mainly for their prognostic roles in the carcinogenesis [7, 8, 21–30]. There are many questions in the current literature about the role of the HER2, PTEN, p53, EGFR in human development. Therefore, our goal is the comparative examination of the expression of these markers in the digestive tract of 12–24-week-old fetuses.

Materials and Methods

We studied 18 post mortem fetuses of 9–24 weeks from the Laboratory of Histopathology, Emergency County Hospital of Tîrgu Mureş, Romania. Our study is approved by the Ethical Committee of the University of Medicine and Pharmacy of Tîrgu Mureş. The age of the fetuses was established based on the crown-rump length, and together with their gender, it was used to classify the fetuses into the groups shown in the table below (Table 1).

Table 1 – *Grouping of the fetuses based on their age and gender*

Age [weeks]	Males	Females	Total
9–12	–	1	1
13–16	4	1	5
17–20	3	2	5
21–24	2	5	7
Total	9	9	18

We performed the dissection of the fetuses and collected samples from the esophagus, stomach, small

and large intestines. These samples were fixed in 4% formaldehyde overnight, and then embedded in paraffin. The 5-µm thick sections obtained from the paraffin embedded resection tissue specimens were routinely dewaxed and rehydrated. The endogenous peroxidase was blocked by a 10 minutes 3% H_2O_2 bath. Antigen retrieval was performed by pressurized steam cooking for 20 minutes (Tris-HCl solution, pH 10 for HER2 and PTEN; citrate solution, pH 6 for p53 and EGFR).

Non-specific protein binding was blocked by incubation with LabVision UVBlock for five minutes. We used the following primary antibodies: HER2 (rabbit monoclonal antibody, clone SP3, LabVision), 1:50, 50 minutes; p53 (mouse monoclonal antibody, clone DO-7, Diagnostic BioSystem) 1:100, 50 minutes; PTEN (mouse monoclonal antibody, clone PTN-18, Diagnostic BioSystem) 1:25, 4^0C overnight and EGFR (mouse monoclonal, Ab-10 clone 111.6, LabVision, Fremont, CA, USA), 1:25, overnight 4^0C. The UltraVision LPValue Large Volume Detection System HRP Polymer (LabVision) and DAB chromogen was used for detecting the primary antibodies. This was followed by Hematoxylin staining.

In order to quantify and compare the HER2 immunoexpression, we used the grading system described in the literature for digestive tract tumors: 0 (no staining), 1 (weak and not complete staining <10%), 2 (moderate and complete staining >10%), 3 (strong and complete staining >10%) [31]. In case of p53 and PTEN, based on the percentage of stained cells, we considered negative staining if 0% of the cells were labeled, weak staining if 0–25% of the cells were labeled, moderate staining if 25–50% of the cells were labeled and strong staining if 50–100% of the cells were labeled. We used the sample recommended by the manufacturer as positive control, and the samples not labeled by the primary antibody were used as negative controls. For EGFR, we used the intensity scale recommended by the manufacturer: (1+) weak, less than 10% of the cells showing a membrane reaction, located at the apical pole of the membrane and not surrounding the cell, (2+) moderate, more than 10% of the cells showing a membrane reaction, located at the apical and lateral aspects of the cell membrane, (3+) strong, more than 10% of the cells showing a membrane reaction completely surrounding the cell.

Results

HER2 expression

The expression of HER2 in the esophagus was poor (1+) in the 9–12 weeks period, then it increased to moderate (2+) intensity in the 13–16 and 17–20 weeks period. At the beginning of the 21–24 weeks period, the expression was strong (3+), then at the end of the period it decreased to poor intensity (1+) (Figure 1a). We observed a specific luminal enhancement.

HER2 immunoexpression in the gastric mucosa appears only in the 13–16 weeks period, then on week 16 the intensity becomes moderate (2+), and at the end of the 21–24 weeks period it reaches strong intensity (3+) (Figure 1a). Similar to the esophagus, the luminal enhancement was also present in the gastric mucosa.

In the small intestine, we found moderate (2+) HER2 expression during the 13–16 weeks period, which decreased to poor (1+) intensity by the end of this interval. This poor intensity was also present in the 17–20 weeks period. At the beginning of the 21–24 weeks period, the expression increased to strong (3+) intensity, but it disappeared at the end of the period (Figure 1a). The luminal enhancement was present.

Similar to the small intestine, HER2 expression was absent in the mucosa of the large intestine between weeks 9 and 12, then at the beginning of the 13–16 weeks period it had moderate (2+) intensity. Later it decreased to poor (1+) intensity. At the end of the period, it became again moderate (2+). During the 17–20 weeks period the intensity remained moderate (2+), then at the beginning of the 21–24 weeks period it became strong (3+) (Figure 1b) and it disappeared at the end of the period (Figure 1a). Luminal enhancement was also observed in the large intestine.

EGFR expression

During the 9–12 weeks period, we observed a poor (1+) expression in the apical membrane of mucosa cells of the esophagus, which at the end of the 13–16 weeks period increased to a moderate (2+) intensity. At the beginning of the 17–20 weeks period the expression is characterized by poor (1+) intensity, but it becomes moderate (2+) again at the end of the period. At the beginning of the 21–24 weeks period, the expression is strong (3+), then to the end of the period it decreases to poor (1+) intensity (Figure 2a).

In the stomach, we found poor (1+) expression during the 9–12 weeks period, which remained constant in the 13–20 weeks period. At the beginning of the 21–24 weeks period, the intensity of the expression was moderate (2+), then it became strong (3+) at the end of the period (Figure 2a).

The EGFR expression in the small intestine appeared only at the middle of the 13–16 weeks period with poor (1+) intensity and remained constant until the end of the period. In the beginning of the 17–20 weeks period it was moderate (2+), at the end of the period it became strong (3+). During the 21–24 weeks period the expression decreased to poor (1+) intensity (Figure 2a).

In the large intestine, we did not find EGFR expression until the middle of the 13–16 weeks period. When it appeared, we observed poor (1+) intensity, which remained constant until the middle of the 17–20 weeks period. After this, until the end of the 17–20 weeks period the expression increased to moderate (2+) intensity. At the beginning of the 21–24 weeks period, we observed further increase (3+) of the expression (Figure 2b), which was followed by the disappearance of the expression at the end of the period (Figure 2a).

P53 expression

In the esophagus and the stomach, we found strong expression during the examined periods (Figure 3a). In the small and the large intestine, we found a uniform strong expression until the 21–24 weeks period. Subsequently, the expression depends on the localization: stronger in the crypts than in the other parts of the epithelium (Figure 3b).

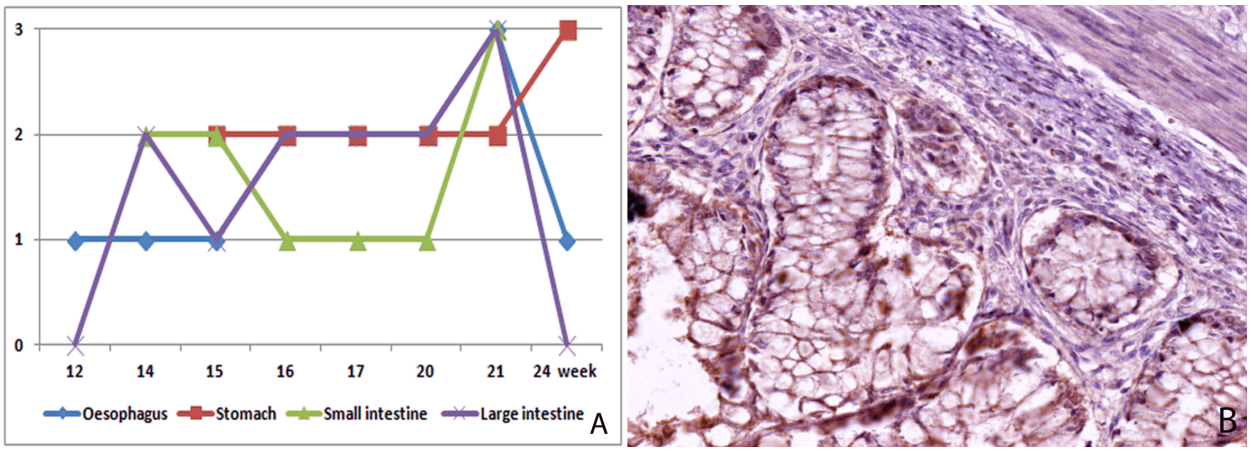

Figure 1 – *(a) The modification of the HER2 expression in the gastrointestinal tract; (b) HER2 expression (3+) in colon, week 21, ×200.*

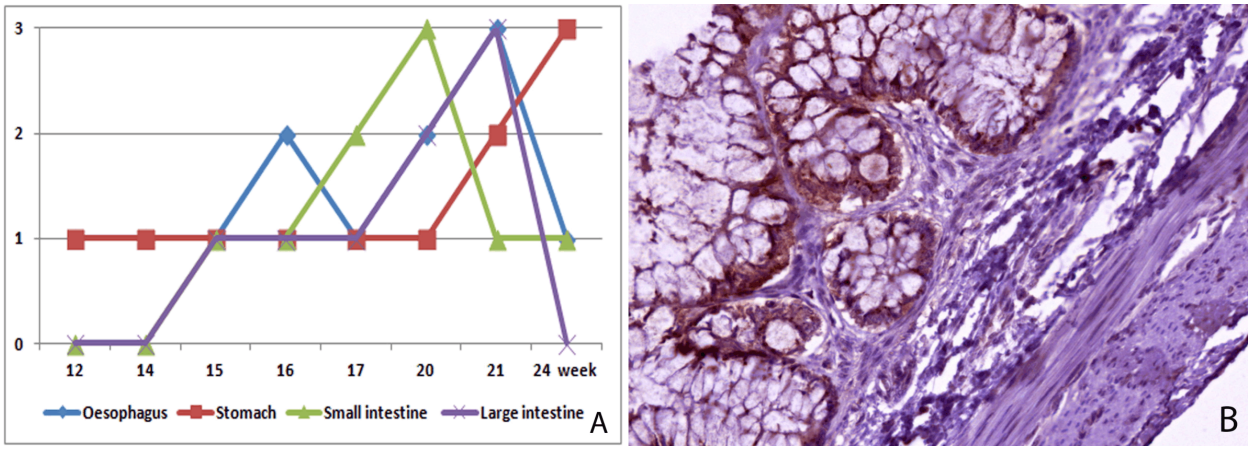

Figure 2 – *(a) The modification of the EGFR expression in the gastrointestinal tract; (b) EGFR expression (3+) in colon, week 21, ×250.*

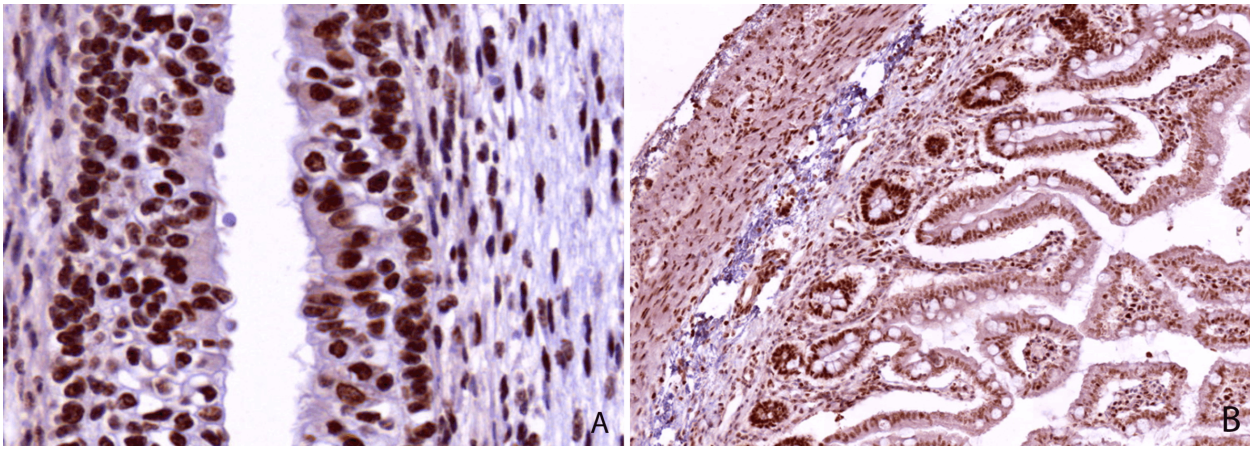

Figure 3 – *P53 expression: (a) in esophagus, week 12, 400×; (b) in small intestine, week 21, ×100.*

PTEN expression

PTEN expression in the esophagus during the 9–12 weeks period is stronger (2+) in the superficial cells than in the deeper layers of the epithelium (1+) (Figure 4a). PTEN expression increases progressively in the deeper layers of the epithelium until the middle of the 13–16 weeks period. At the end of the period, we found moderate (2+) expression in the whole of the epithelium, which subsist also in the 17–20 weeks period. However, during the 21–24 weeks period, we observed a strong (3+) PTEN expression (Figure 4b).

We found moderate (2+) PTEN expression in the stomach during the 9–12 weeks period. During the 13–16 and 17–20 weeks periods, the expression had strong (3+) intensity (Figure 4c). Moderate (2+) expression was observed during the 21–24 weeks period (Figure 4b).

During the 9–12 weeks period, we found poor expression in the small intestine. At the beginning of the 13–16 weeks period, the expression increased to moderate (2+) intensity, then starting from the middle of the period until to the end of the 21–24 weeks period we found strong (3+) intensity (Figure 4d).

In the large intestine, we observed poor (1+) expression until the middle of the 13–16 weeks period. From the middle of the period, until the end of the 21–24 weeks period, we found constant, moderate (2+) expression (Figure 4b).

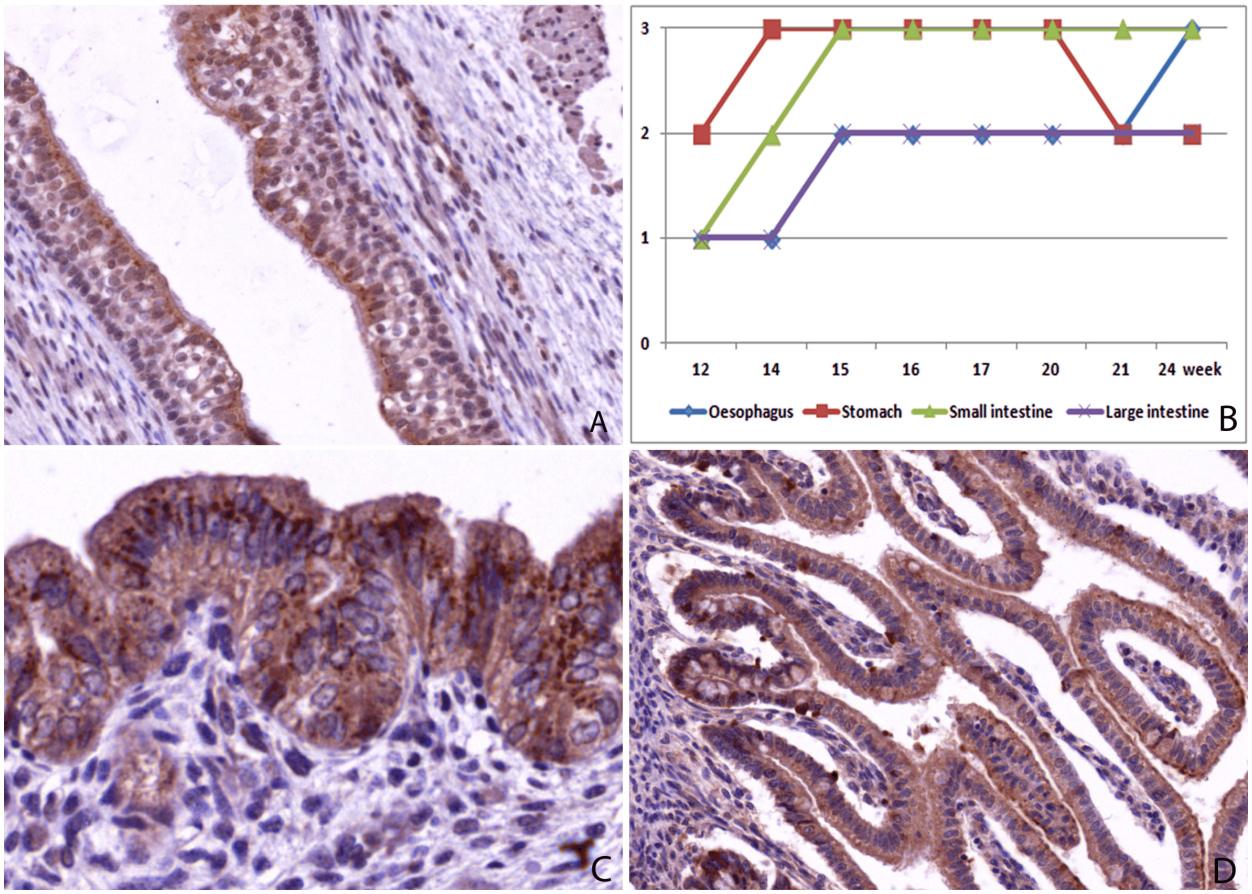

Figure 4 – ***(a) PTEN expression (2+) in esophagus, week 15, ×200; (b) The modification of the PTEN expression in the gastrointestinal tract; (c) PTEN expression (3+) in stomach, week 15, ×400; (d) PTEN expression (3+) in small intestine, ×200.***

Discussion

By activating a complex intracellular signaling pathway, the HER2 receptor induces cell proliferation, differentiation and migration, which is blocked by PTEN. At the same time, p53 causes apoptosis, and stops the cell cycle by stimulating PTEN [10, 13, 32]. Knowing the multi-faceted role in carcinogenesis of these markers and considering the paucity of literature data regarding their role in development, our study aims to examine HER2, PTEN, EGFR and p53 immunoexpression in different segments of the digestive tract.

In the 9–12 weeks period, we found HER2 expression only in the esophagus, whilst in the other segments it appeared only in the 13–16 weeks period. During the 13–20 weeks period, the HER2 expression was characterized by luminal enhancement and varying (poor–moderate) intensity, and subsequently, during the 21–24 weeks period, it was characterized by a sudden increase of the intensity, which was followed by the disappearance of expression. Most of the studies in the literature examined HER2 expression during embryonic development. High HER2 expression was observed in epithelial structures of E14 and E16 rat embryos [7]. Extensive HER2 expression was observed in all three embryonic germ layers of human embryos, thus demonstrating the important role of HER2 in embryogenesis [8]. Contrary to our results, Quirke *et al.* (1989) found positive HER2 expression until week 12 of development in all segments of the human digestive tract, and they also described the luminal enhancement [8].

Expression of EGFR was poor in all segments of the digestive tract during weeks 13–16. At the 17–20 weeks period, it became moderate, then at the beginning of the 21–24 weeks period, its intensity increased, and subsequently decreased. The gastric mucosa was an exception because we observed increased expression at the end of the 21–24 weeks period also. Literature data about the first appearance of EGFR expression in the digestive tract is diverse. Similar to our results, Oliver (1988) observed poor expression in the villi of the digestive epithelium during week 16, which increased during weeks 18 and 19. Also according to their findings, EGFR expression in the stomach first appears during week 17, and shows poor intensity. During weeks 18 and 19, similarly to the other segments of the digestive tract, its intensity increases [5]. Based on the above, Quirke *et al.* (1989) suggested that EGFR has a less important role in early fetal development than HER2, because EGFR expression cannot be observed in the digestive tract before week 16 [8].

According to Hormi & Lehy (1998), a moderate EGFR expression is observed in the epithelium of the esophagus as early as the 9–10 weeks period, then it disappears at the beginning of the 14–18 weeks period.

According to their findings, a poor expression appears at the end of the period, then it increases to moderate intensity by the end of the 20–24 weeks period. In case of the stomach, EGFR expression first appears during the 14–18 weeks period, and there are differences in the way it changes in different gastric areas. Similar to our findings, an increase in of gastric EGFR expression was observed during the 20–24 weeks period. However, in the small intestine, the intensity was poor in the villi and crypts during the 14–18 weeks period, then in the second part of the 20–24 weeks period strong EGFR expression was described. The differences between our findings and literature data regarding EGFR expression are possibly due to the use of different primary antibodies and different scoring systems [4].

We observed strong p53 expression in all segments of the digestive tract during the 9–21 weeks period, and subsequently p53 expression increased in the basal third of the small and large intestine crypts. According to literature data, p53 expression positively correlates with high proliferation activity in the embryonic period during organogenesis, then it decreases in human fetal tissues after the week 10 [33]. During the early stages of mouse embryonic development, p53 expression was observed in all of the tissues, but in later stages it becomes tissue specific (brain, liver, lungs, thymus, intestines, salivary glands, kidney) [11, 12]. In the stage of organogenesis and histogenesis it is heterogenic [11]. In later stages of organogenesis, p53 levels decrease, and it is hardly detectable in fully developed tissues [11, 12]. The multilayered endodermal epithelium of a 14.5 *post coitum* (*p.c.*) day mouse embryo showed a strong intensity p53 expression, then on the 16.5 *p.c.* day p53 expression increased in the intestinal crypts compared to the epithelium of the villi [11].

PTEN expression shows luminal enhancement in the esophagus during the 9–12 weeks period, then in the 13–16 weeks period, it has a uniformly dispersed moderate expression throughout the epithelium, and in the 21–24 weeks period it has a strong expression. Gastric PTEN expression is maximal during the 13–20 weeks period, and later it decreases to moderate intensity. In the small intestine, similar to the esophagus, the expression progressively increases during the 9–20 weeks period, and it reaches its maximum in the 21–24 weeks period. In the large intestine, a progressive increase was observed in the studied intrauterine periods.

PTEN expression was demonstrated in many tissues during early stages of embryonic development. In later stages, the expression is tissue specific [19]. In case of the murine digestive tract, PTEN expression was noticed in early stages of development, then it was restricted to the epithelium of the small and large intestine, but it was present also in the gastric and esophageal epithelium [14]. Based on the literature data PTEN expression was relatively high in the human fetal esophageal epithelium, compared to earlier stages of development. Poor PTEN expression was observed on days 37 and 41 in the stomach. On week 17, PTEN expression was poor also in the epithelium of the small and large intestine [19].

Conclusions

Our results complete the scarce data found in literature about the expression of the studied markers in the development of the digestive tract. Poor HER2 and PTEN expression appears as early as the 9–12 weeks period in the digestive tract, whilst EGFR expression appears only during the 13–16 weeks period. The expression of HER2 and EGFR decreases in the 21–24 weeks period and then disappears. The expression of p53 is strong until week 21, and then it is restricted to the deeper layers of the epithelium, whilst the expression of PTEN progressively increases in the epithelial cells during the examined period and becomes uniform.

Conflict of interests

The authors declare that they have no conflict of interests.

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