

IMMUNOHISTOCHEMICAL FEATURES IN THE DEVELOPMENT OF THE FETAL GASTROINTESTINAL TRACT

Abstract

Introduction: The digestive tract extends from the distal end of the pharynx to the anus. It has many anatomical and functional segments. In these segments takes place the series of events linked to the digestions and absorption. So the digestive tract has role in the digestion and absorption of the nutrients, propulsion of these and the elimination of the waste products.

The epithelium is characterized by a very high cellular turnover. The epithelial cells are renewing by well defined cellular production and migration at 3 – 5 days. This system is sustained by the balance between proliferation, differentiation and apoptosis at the level of the epithelium. These processes are well defined from embryonic age.

The apoptosis is the programmed cell death with central role in tissue morphogenesis and homeostasis in the development and postnatal period. The proliferation is the sum of the changes at the level of the genome which result in cell division and maturation of the cells. The tissue homeostasis depends on both of apoptosis and proliferation.

The turnover of the gastrointestinal epithelium is strictly regulated at molecular level by numerous intracellular signalling pathways: Wnt pathway, PTEN-PI3K-Akt pathway, BMP pathway, Notch pathway, Hedgehog pathway. One of the most important pathways is the PTEN-PI3K-Akt pathway. The activation of the surface receptors (HER2 and EGFR) with tyrosine-kinase activity, results in the activation of the PI3K, which phosphorylates PIP2 to PIP3. PIP3 phosphorylates the protein kinase B through PDK1. Thus the cellular proliferation, differentiation, migration and RNA translation are activated. The only regulator of this pathway is PTEN, which dephosphorylates the PIP3 to PIP2, thus reducing the level of the PI3K's substrate, so reducing the Akt activity, cellular proliferation, differentiation, migration and RNA translation. Any modification in the structure of the DNA activates p53. p53 activates PTEN so it is negatively regulating the cell cycle.

The Hirschsprung disease is one of the most common malformation of the colon. It is characterized by a error in development and migration of the nerve ganglions in the enteric nervous system. NSE (neuron specific enolase) is a marker for the enteric nervous system. The expression of these marker is present in the adult enteric nervous system also.

Histologically, the gastrointestinal tract is developing through three periods, and from rostral to caudal. In the first 14 weeks develops the unique histological structure of each segment, between weeks 14 and 30 appears additional histological structures, and in the third period the structures developed in the previous two are growing.

Objectives: We studied the relation between the growth markers HER2, EGFR and PTEN, the balance between apoptosis and proliferation, p53 and Ki-67, in the gastrointestinal tract, and the relation between the development of the enteric nervous system and the muscular layer of the gastrointestinal tract, NCAM and NSE.

Materials and methods: We studied fetal esophagus, stomach, small and large intestine tissue samples using immunohistochemistry. We preselected samples of 35 fetuses but could only use 18.

Results: The EGFR expression appears later in the small and the large intestine, and increases progressively in the epithelium of the gastrointestinal tract until the week 21, then it decreases until the end of the period studied by us. It is weak in the 13 – 16 weeks period in each segment of the gastrointestinal tract, in the 17 – 20 weeks period it becomes moderate, and at the beginning of the 21 - 24 weeks period it is strong and disappears to the end of the period. The HER2 expression is increasing in

the epithelium of the gastrointestinal tract until week 21, after which it decreases. In the 9 – 12 weeks period it appears in the esofagus. In the other segments it appears only in the 13 – 16 weeks period. The expression in the 13 – 20 weeks period is characterized by luminal accentuation. In the 21 – 24 weeks period the expression is increasing after which is decreasing and disappearing. In the 9 – 12 weeks period the PTEN expression in the esofagus is characterized by luminal accentuation, beginning with the 13 – 16 weeks period it becomes moderate and uniform throughout the thickness of the epithelium and is increasing in the whole gastrointestinal epithelium until week 15, then it becomes constant in the period studied by us.

The p53 expression in our study is strong in each segment until week 21. After week 21 the expression is present mainly in the inferior 1/3 of the intestinal crypts. The Ki – 67 expression is more accentuated in the beginning of the period in all of the gastrointestinal tract's segments. Then it decreases more in the esofagus and large intestine and less in the stomach and ileon. In the 9 – 12 weeks period the proliferation index is the highest in the esofagus and stomach. In the 13 – 16 weeks period it increases in the ileon and large intestine, then until the week 24 there is a decrease in all of the gastrointestinal tract's segments, which is more accentuated in the esofags and large intestine. The Ki – 67 expression negatively correlates with the fetal age.

The NCAM expression is present in the mienteric plexus beginning with the 9 – 12 weeks period. In this period we observed the NCAM expression in the circular muscular layer in the stomach, ileum and jejunum and colon. In the weeks 20-21 the NCAM expression is present in the internal side of the circular muscular layer. The intensity of the expression then decreases, following the path of the migratin nerve ganglions. Thus the NCAM expression initially appears in the mienteric plexus and migrates to the internal margin of the circular muscular layer. The NSE expression is specific for the cells of the fetal enteric nervous system. It appears in the cells of the fetal enteric nervous system in the 9 -12 weeks period in all of the segments of the gastrointetinal tract. In the week 21 the groups of the gangloin cells are well contured. The dimension of the mienteric plexus is positively correlated to the fetal age. The biggest modifications in the dimensions of the mienteric plexus are at the level of the ilon and colon.

Discussion: The proliferative processes are highlighted until week 21 of the prenatal period. Which is shown by the increasing of the expression of HER2, EGFR until this week. These proliferation processes are under the control of p53 which is expressed uniformly in the epithelium of the gastrointestinal tract. Also in this period the expression of Ki – 67 is higher. The PTEN expression is constant. In the esofagus it is mainly present in the cytoplasm of the superficial epithelial cells, not in the deep ones where are the proliferation processes. After week 21 the proliferating processes are reduced and the differentiation and growth processes are highlighted. This is shown by the decrease of HER2, EGFR, Ki – 67. The PTEN expression is constant, which shows us the regulation of proliferation in negative direction. The p53 expression is more intense in the zones with high proliferative activity.

Conlusions: Our results shows that in the development of the gastrointestinal tract the processes of growth, maturization and proliferation or highlighted until week 21, then the processes of differentiation are highlighted. These processes have a rostral – caudal direction throughout the digestive tract. Our morphometric and immunohistochemistry observations sustain the cranio-caudal development of the circular muscular layer and the enteric nervous system and underline the fact that NSE and NCAM are specific markers of the enteric nervous system.