ABSTRACT

The peripheral nervous system (PNS) is derived from neural crest cells, which are present only in vertebrates. The PNS development is initiated at the early stages of embryogenesis, when the neural crest is formed, and from which the neural crest cells (NCCs) delaminate and migrate through the developing organism. The formation of the PNS is a multi-stage and complex process dependent on many mechanisms at the cellular and molecular levels. They include the activity of transcription factors, cascades of genes expression, the functioning of cellular proteins, and interactions of cells with the extracellular environment. During the formation of the PNS there comes up among others to the intensive migration of neural crest cells, formation of dorsal root ganglia, axonal extension by sensory neurons, innervation of the target site, migration, and differentiation of glial cell precursors, which are also the source of pigment cells - melanocytes. In all these processes cell adhesion and migration, the processes dependent on the interaction of cell with the extracellular environment, play an important role. These interactions activate intracellular signaling pathways and influence the reorganization of cellular structures related to the actin cytoskeleton.

The functioning of actin cytoskeleton-based structures, and migration and adhesion processes, in general, are dependent on many proteins. The extracellular factor, which plays a key role in these processes is laminin. Among cellular proteins associated with those processes allowing cell to respond to the presence of laminin in the cell environment are gelsolin (GSN), non-integrin laminin receptor (LamR), and integrin-linked kinase (ILK). Studies conducted by our group have shown that gelsolin forms complexes with ILK and LamR in human skin melanoma cells - transformed melanocytes. Therefore, it has been decided to investigate what role these proteins play in forming the PNS during chicken embryonic development, because it seems that they play an important role in melanoma cells and melanocytes originate partially from the developing PNS. It was decided to work on chicken embryo, as the formation of PNS and melanocytes in chicken embryo is similar to their genesis in humans.

In the first stage of the research, the pattern and level of *RPSA* and *ILK* expression during the chicken's embryogenesis were determined. These parameters for *GSN* have already been investigated by our group. It has been shown that both *ILK* and *RPSA* are expressed already at early stages of chicken embryogenesis. Furthermore, analysis of the expression pattern of the studied genes indicates their strong expression in tissues and organs associated with the formation of PNS and melanocytes.

Then, the effect of *RPSA*, *GSN*, and *ILK* expression silencing on the trunk neural crest cell *in ovo* was determined by analyzing the delamination of NCCs and their migration, as well as their distribution and the development of their derivatives. Silencing of the *RPSA* expression has been shown to cause abnormalities in the delamination of neural crest cells and silencing of *GSN* expression in homing specific locations for their migration pathways. However, silencing of the tested genes did not impair the development of neural crest cell-derived cells.

The subcellular localization of LamR, GSN, and ILK in neural crest cells and on their surface was also investigated. In addition, their effect on the delamination and migration of neural crest cells dependent on laminin-1 and its peptides was determined *in vitro*. For this purpose, the functioning of the studied proteins was disturbed by the application of antibodies directed against them. The data confirmed the presence of all three proteins in neural crest cells. Only GSN was not present on the outer site of the plasma membrane of these cells and did not affect their migration. However, GSN was secreted into the culture medium. Whereas LamR and ILK turned out to be significant for these processes. Although all three proteins influenced the ability of analyzed cells for the formation of focal adhesions, only GSN, and LamR impacted the formation of filopodia, structures involved in the migration and adhesion.

Subsequently, the localization and the role of LamR, ILK, and GSN in the functioning of the developing dorsal root ganglia was determined, focusing on the parameters that determine the axonal extension and the properties of Schwann cell precursors to migrate along neurites. For this purpose, the activity of the tested proteins was disturbed by the application of antibodies directed against them. All tested proteins were present in sensory neurons and glial cells. In addition, GSN was the only protein that was also present in the conditioned medium. LamR, ILK, and GSN have been shown to play a role in neurite elongation, their directional movement, and the migration of Schwann cell precursors in a different manner. Furthermore, these proteins are involved in the reorganization of actin in the growth cones filopodia - structures involved in the neurite's movement and navigation.

In conclusion, the results presented allow to draw a conclusion saying that GSN, LamR, and ILK are involved to varying degrees in laminin-1-dependent formation of the PNS during chicken embryonic development. The role of these proteins in studied processes has not been investigated in the chicken embryo so far, so the results presented here bring new knowledge about chicken embryogenesis in the context of the biological properties of studied proteins.