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POINT OF VIEW

Melanoma and Cancer Development from the Viewpoint of the Work of the Morphofunctional Zones

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Abstract

Proliferation and differentiation of cells occur in the morphofunctional zones, in the electric field excited by 12 pairs of mother and daughter cells, produced by cambial cell division. Thus, in the daughter cells, the Src SH2 domain is essential for both cytoskeleton formation and tyrosinase activation. If the conditions for strengthening the tyrosinase activity are created in an organism, the amount of the Src participating in the cytoskeleton construction process can decrease to a critical level, which will culminate in the development of a malignant tumor. If the action of a stimulating factor is strong enough, the malignant cells will begin to proliferate at the melanocyte stage, and a melanoma will be formed. If the action of the factors is long but not strong, only the more remote descendants of the daughter cells will proliferate, and a cancer will be formed. Therefore, to assure the normal differentiation of the malignantly changed daughter cells, the action of the tyrosinase needs to be blocked. Thus, all the SH2 domains are utilized in cell cytoskeleton construction.

Keywords: *morphofunctional zone; tirosinase; melanoma; cancer; glutathione.*

In our previous studies, the scheme of cell proliferation and differentiation in the system of morphofunctional zones was presented [1, 2]. There, the decrease in the number of the cambial cells in a zone to the critical level was shown to lead to the development of a malignant tumor. However, it was not clear why in one case a melanoma was formed, whereas in the others a cancer was formed. Therefore, we summarized our own experimental material that allowed us to prove theoretically, the emergence of such a malignant nosology and the possibility of the treatment.

Proliferation and differentiation of cells occur in the morphofunctional zone, which contains two subunits, containing 12 cambial cells each. During the cambial cell division, mother and daughter cell pairs are formed. The mother cell is in close proximity to a basal membrane in which the growth factors with spastic action (like TGF-β) get collected. Therefore, the genome having a negative charge, constricts more strongly in the mother cell, than in the daughter cell. This leads to the redistribution of the superficial charges between two of these cells and produces some electric field. The division of the cambial cells in the subunit zone does not happen simultaneously. The first six cells divide at first, followed by the other six in the same subunit zone. The daughter cells formed during the division of the first six cambial cells do not elongate in the electric field, i.e. they do not get exposed to differentiation. Only during the division of all the 12 cambial cells of the same subunit, the differentiation of the daughter cells is observed. Therefore, the electric field formed by the six mother and daughter cell pairs does not bring about the differentiation of the daughter cells.

Stroma greatly influences on the differentiation of the daughter cells. The same morphofunctional zones in which the cambial cells divide simultaneously with the epithelial cambial cells are observed in it. The function of the stroma is to cause a relaxation of the epithelial cell cortex, which is constricted due to its own growth factors, and therefore the epithelial daughter cells have an opportunity to stretch in the electric field and to be differentiated.

Two key proteins participate in the realization of the process of the cell differentiation, which occurs in the morphofunctional zone, viz., tyrosine kinase Src and RhoA (one of the small G-protein). The stromal daughter cells,

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under the influence of their growth factors over the epithelial cells, activate the Src kinase SH3 domain within them through several intermediaries. Then, the Src phosphorylates the p190RhoGAP protein, which inactivates the RhoA, resulting in a weakening of the actin-myosin interaction, which in turn leads to a relaxation of the epithelial cell cortex and permits the possibility of their stretching in the electric field. However, the cortex relaxation is only a preparatory stage to the actual differentiation. A system of microtubules and their intermediate filaments stretching the nucleus will cause the untwisting of certain loci of the chromosomes responsible for various differentiations, which is an essential step. These microfilaments have been shown, in this study, to develop in the cells during the stretching of the weakened epithelial cell cortex in the electric field. Therefore, the cell stretching leads to an active splitting off of the exocytose bubbles from the Golgi complex membranes resulting in them being inserted into the plasma membrane as well as the outflowing of the inactive Src localized on these membranes. Near the Golgi complex, at the centrosome, the microtubules are formed, which nucleation occurs in large protein complexes, consisting of Src, PI3K (phosphoinosite-3-kinase) and γ–tubulin. The Src interacts poorly with γ-tubulin as it has a low affinity for it. The active PI3K, however, interacts with the γ-tubulin, phosphorylating it and readying it for interaction with the Src. Thus, the inactive Src associates with the active phosphotyrosine γ-tubulin site by means of the SH2 domain very essential for the stable interaction of the tubulin dimers through the SH2 group. Thus, the kinase site of the SH2 domain becomes active. In this regard, the microtubule system and the intermediate filaments are formed.

Therefore, the influence of the fibroblast growth factors on the daughter epithelial cells causes the activation of the Src SH3 domain in them. It is essential for their cortex relaxation;, and during the stretching in the electric field, the activation of the Src SH2 domain necessary for their direct differentiation takes place. Thus, the Src expression is increased in the epithelial cells during their stretching, although the RhoA remains moderately above the Src.

Simultaneously, with the stretching, black-brown pigment granules and a dense network of thin processes in the cytoplasm of a daughter cell appear, indicating the activation of tyrosinase and melanogenesis occurring there [3]. If the daughter cell stretching stage is missing, as is observed soon after cambial cell division, tyrosinase staining is not observed. Therefore, melanogenesis begins at the time of cell stretching accompanied by the expression of the Src SH2 domain in it, i.e. this domain directly participates in the transcription and tyrosinase activation. In reality, in the epithelial cells the RhoA is moderate above the Src, which induces this cell stretching and the untwisting of the DNA loops closer to the telomeres and defines their epithelial differentiation [1]. Therefore, at the same sites there is a possibility of the transcription of the genes coding tyrosinase. Tyrosinase activation requires its phosphorylation, which occurs in the region connected with the Golgi complex [4]. Considering the fact that the tyrosinase activation happens at during stretching, it is possible to conclude that phosphorylation is performed at the expense of the active Src, which is located here. Therefore, the degree of the expression of the Src SH2 domain defines the transcription and activation of the tyrosinase. Therefore, various constitutional levels of the Src kinase expression in the organism will define the prevalence of eumelanin or pheomelanin in an individual. Thus, the normal constitutional level of Src expression will provide a large amount of active tyrosinase which will catalyze the hydroxylation of tyrosine in the DOPA and DOPA oxidation in the DOPA-quinone, leuco-DOPA-chrome. Besides, the high tyrosinase activity will oxidize the sulfhydryl groups of the proteins, thereby blocking the proteins, preventing them from joining to the forming melanin. Really, if the protein had joined in, it would have changed the configuration of the melanin formed and would have weakened its interaction with the enzyme. This lack of connection with the proteins enhances the chances of further formation of the 5, 6-dihydroxyindole, the polymerization of which leads to melanin formation.

Pheomelanin is formed in individuals who possess a lower level of the constitutional Src kinase expression, than in those having eumelanin. The lesser quantity of the active tyrosinase, which can oxidize the tyrosine only until the DOPA-quinone stage, will thus be formed. Cysteine joins the DOPA-quinone then via the sulfhydryl group because this small quantity of tyrosinase cannot oxidize and inactivate this group. Cysteine joining in will change the DOPA-quinone configuration, which will halt further melanogenesis. Therefore, the 5-S-cysteinyl-DOPA, which is formed, is a monomeric unit of the pheomelanin polymer. It is interesting to note that the melanosomes containing eumelanin are more elongated in shape than those with the pheomelanin, which proves that the genetically programmed Src activity in individuals with the pheomelanin is less expressed. This in turn leads to the activation of the RhoA, which strengthens the spasmodic ability of these structures. Also, the incorporation of the tyrosinase into the melanosome during the formation of the eumelanin happens during the later stages of melanosome development, than during the pheomelanin formation. During the normal constitutional activity of the Src, which does not , sharply reduce the RhoA, there will be regular splitting of the premelanosomes from the smooth membranes of the granular endoplasmic reticulum. Therefore, the tyrosinase formed will get incorporated into the melanosome at a certain stage of maturity. In the case of reduced constitutional Src activity, the RhoA activity will increase which will lead to a strengthening of the spasm of the endoplasmic reticulum membranes and a delay in the premelanosome splitting. In this regard, the tyrosinase formed will get incorporated into the melanosome, during the earlier developmental stages.

Thus, in the epithelial tissue, the cambial cell division passes through the melanocyte (daughter cell) formation stage. Thus, the Src kinase SH2 domain, which is highly essential for the formation of the microtubules and intermediate filaments, participates not only in the assembly of the cytoskeleton and cell differentiation, but also in the production and activation of the tyrosinase, necessary for melanogenesis.

In the process of the melanocyte (daughter cell) motion in the direction of the basal cells, the RhoA activity due to

the microenvironment increases within it, and the degree of Src expression falls. Therefore, the melanocytes in the basal layer will lose their tyrosinase activity. Thus, the action of the various agents, either strengthening or weakening the tyrosinase synthesis depends upon the link to which their action is directed – to the Src or the RhoA.

It has been mentioned earlier by us that cell proliferation and differentiation occurs in a morphofunctional zone on which various factors viz., physical, chemical, hormonal, traumas and age, exert their influence [2]. If, during the action of these factors, the number of cambial cells decreases to the threshold level (6 cells), the differentiation of the daughter cells is absent and a malignant tumor is formed. Thus, in the daughter cells an insufficient activation of the Src SH2 domain is observed, essential for their differentiation. With age, the number of the cambial cells in each zone subunit gradually decreases and reaches seven cells in individuals above 75 years of age. This is also close to the threshold level and increases the risk of the emergence of a malignant tumor in the elderly. Therefore, the action of the various carcinogenesis-inducing factors is seen as a result of the decreased expression of the Src SH2 domain in the daughter cells to a critical level which is insufficient for the cell cytoskeleton development, and for this purpose there cannot be an obligatory decrease in the number of cambial cells. Several tumors develop in individuals who showed no age-related decrease in the cambial cell number.

Therefore, at the normal cambial cell number (12 cells), the tyrosinase synthesis can increase under the influence of many factors. Under these conditions an increased utilization of the Src will be observed for the tyrosinase phosphorylation which will, in turn, reduce the participation of the Src SH2 domain in cytoskeleton formation. If the portion (amount) of this domain decreases to the critical level, differentiation will not occur and a malignant tumor will be formed. This occurs more frequently in individuals with a low constitutional level of Src kinase activity. Therefore, individuals with pheomelanin, more often fall ill with a melanoma and a cancer of skin. If the factor activating the tyrosinase is very strong (for example, powerful UF radiation), then during the phase of a considerable decrease in the SH2 domain, the expression of the RhoA will sharply rise in the cell cytoskeleton. We have mentioned this earlier that the ratio of the Src and RhoA expression defines not only the differentiation, but also cell proliferation. Cells proliferate only in cases when the degree of the RhoA expression in them exceeds that of the Src more than moderately [2]. Src kinase inactivates the RhoA, but quite high expression of the RhoA protein, will ensure that the most part of the RhoA remains active. As a result, more formins and microtubules ensuring mitosis are formed. Therefore, the daughter cells at the melanocyte stage, i.e. the earliest stages of the development, will begin to actively proliferate leading to the development of a melanoma (Figure 1). If the action of the activating factors is neither strong nor sufficiently long, the activity of the SH2 domain will gradually decrease. Therefore, the malignancy and active proliferation will be evident in the more remote descendants of the daughter cells, which do not already possess the tyrosinase activity, and a cancer will be formed. The period of the greatest estrogen activity, when the influence of the estrogen on the tissue increases, is especially dangerous, because the estrogens directly activate the Src kinase and thus in turn increase the tyrosinase activity of the cells.

Thus, the daughter cells (melanocytes), produced soon after the cambial cell division and possessing tyrosinase activity, actively proliferate to produce a melanoma. In cancer, the more remote descendants of the daughter cells, which have lost their tyrosinase activity, intensively divide. However, in both these cases the source of malignant regeneration is the daughter cells which possess the tyrosinase activity and which represent the so-called tumor stem cells.

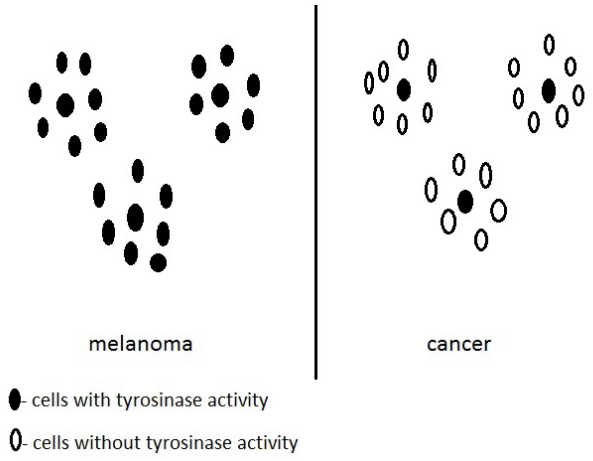

Figure 1.

Melanoma and cancer development concerning tyrosinase activity of daughter cells

Therefore, in tumor tissue, the degree of the Src SH2 domain activation is insufficient for differentiation . Therefore, to remedy such a situation, it is necessary to strengthen the expression of this domain in these cells. As this domain participates simultaneously in the tyrosinase activation and in cytoskeleton construction, it becomes necessary to block the tyrosinase; therefore, the entire quantity of the SH2 domains will be utilized for the cytoskeleton construction for the purpose of differentiation. One of tyrosinase blockers is glutathione, containing the sulfhydryl group (SH) at the expense of the cysteine present in its composition. Glutathione is in its restored and oxidized form in the cell. The restoration of the oxidized glutathione occurs due to the hydrogen sources developed during the course of the metabolism in the organism. One of functions of the glutathione is the binding of the cupriferous enzymes. The tyrosinase active center is known to contain two cations of copper, each of which is directed by three gistidine remnants. The imidazole group of the histidine in a molecule of tyrosinase interacts with the copper cations, triggering the functional activity of the enzyme. However, the SH group differs exclusively in its high reactionary ability [5]. Competitively forcing out the imidazole group, the SH group of glutathione will interact with a copper atom, thus

leading to the formation of the mercaptides. However, the glutathione is quite easily restored within the cell, which can lead to its detachment from the tyrosinase active center and the activation of the latter. To support the interaction of the glutathione with the tyrosinase and its blockage, it is necessary to block the restoration of the glutathione by its contact with the various electron-acceptors, for example, iodine, being the most physiologic for the organism. Therefore, the introduction of iodine into the organism will lead to tyrosinase blockage, and all the Src SH2 domains thus will be used only during differentiation. Therefore, the malignantly transformed daughter cells, which are the centers of tumor growth will become differentiated which in turn will cause the termination of the malignant process.

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