Tumor-Specific Blood Serum Factors as Basis of Tumor Dormancy

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Abstract: In the present review, we focus on the importance of blood serum factors for tumor growth *in vivo*. Data from mice experiments indicate the existence of serum factors, which decrease the dormancy of Ehrlich carcinoma cells from 85 to 20%. The impaired production of these factors increases the life span of tumor-bearing animals from 14 days to 120 days. Blocking the production of tumor-specific factors causes the complete regression of already developed Ehrlich carcinoma. These serum factors do not affect the malignant carcinoma cells *in vitro*. We identified serpins as tumor dormancy serum factors. Experimental evidence suggests that serpins are not only essential for tumor growth. Serpins are also involved in the regeneration of normal tissues, such as adipose tissue, recurrence after cosmetic operations (liposuction), inhibiting rejection after liver transplantation, protection of parasitic flat worms living in host tissues and organs etc. We conclude that the inhibition of serum dormancy factor may represent attractive novel strategies for the prevention and treatment of relapsed cancers.

Keywords: Anti-proteases, Ehrlich carcinoma, Proteases, Serpins, Serum proteins, Tumor growth, tumor dormancy.

1. RELAPSE

One can speak about tumor-specific factors to describe various biological effects of blood serum proteins of experimental animal models. As an example, Fisher *et al*. have shown using 8 tumors of various origins that removal of primary tumor nodes resulted in increased mitotic indices of the residual tumor cells [1]. The authors concluded that there is a tumor-specific serum factor, which accelerates tumor cell growth and division. Remarkably, there was an interval of one decade between the first work of these authors [1] and the second investigation reporting on the unsuccessful attempt to identify such a tumor-specific serum factor [2] pointing to the complexity of this phenomenon. It can be assumed that the difficulty to identify such a serum factor might be related to the fact that the surgical removal of a tumor is always connected with narcosis, bleeding and trauma of the tumor-bearing host.

A couple of years ago, we focused on this problem by using an experimental Ehrlich ascites carcinoma animal model. This is a rather old classical tumor model developed in Germany more than one century ago [3]. The tumor line is characterized by its aggressive behavior capable to overcome interlinear immune compatibility barriers [3]. Removal of this tumor is restricted to piercing the peritoneum by a needle and sucking tumor-cell containing ascites fluid. The ascitic tumor cells are freely floating in the ascites fluid and, hence, wounds and contact breaking between tumor and surrounding normal tissue during removal does not take place. Nevertheless, even the minimal trauma caused by removal of ascites tumor cells is sufficient to increase the mitotic activity of the residual tumor cells after 24 hrs. Interestingly, the increase of mitotic activity is in direct relation to the quantity of the removed tumor-cell bearing ascitic fluid. Removal of 1.5 ml ascitic fluid caused an increase of the mitotic index from 15% to 30%, and removal of 5-6 ml to 80% [4, 5]. These dramatic increases of mitotic activities can only be observed 24 hrs after tumor removal. Afterwards, mitosis drops down to initial baseline levels. Therefore, the question about the underlying mechanisms arises. As contact interaction between cells is not a major factor in ascitic fluid. Furthermore, the cell concentration per milliliter does not change after removal of ascitic fluid. Hence, other reasons have to be responsible for this phenomenon and it can be speculated that a certain factor may be developed by tumors in tumor-bearing hosts causing cell-division rates of around 15%. When the ascitic cells are partly removed, the quantity of this factor is sufficient to stimulate residual tumor cells. However, the question still remains, why the mitotic activity of tumor cells decrease to baseline levels after 24 hrs. This period of time is not sufficient to restore the quantity of removed tumor cells by cell growth. A possible explanation might be the existence of a feedback mechanism that curtails the production of a tumor-specific serum factor. Clues for the existence of such a feedback mechanism come from experiments showing that mice generate more immune cells (blood

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leukocytes, peritoneal cells, and spleen cells) 7 hrs after the removal of ascitic fluid. Interestingly, injection of these cell types into healthy mice leads to resistance of these animals towards tumor development [4,5]. Since this phenomenon cannot be observed by other experimental interventions (*e.g*. vaccination against tumor antigens, killing of tumor cells by heat treatment etc.), a specific mechanism can be supposed. If tumor cells are isolated at other time intervals after ascites removal (*e.g*. 3 or 10 hrs), the development of resistance towards growth of Ehrlich carcinoma cells cannot be observed. Having this tight time frame in mind, we developed regimens of immune cell injections leading to a prolongation of the normal survival time of Ehrlich-carcinoma-bearing mice from two weeks to up to three months [6,7]. The data indicate that the Ehrlich carcinoma cells did not lose their malignancy but that growth of malignant cells might be due to the presence of a antidormancy serum factor.

2. PROTEASES AND ANTIPROTEASES (SERPINS)

Previously, we identified this blood serum factor as serpin, which is produced in the liver [8,9]. To understand the underlying mechanism, it is necessary to consider two experimental observations. The first one is that organ- and tissue-associated immune cells contain tissue-specific sets of proteases [10]. It is important to note that proteases can be highly specific. For example a specific 20 kDa protease of *Klebsiella pneumoniae* degrades proteins of foreign species, but not own proteins. Thereby, the bacterium can efficiently distinguish between “own” and “foreign” - a feature that it is otherwise known from the immune system of higher organisms [11]. Many mammalian proteases have molecular weights of up to 100 kDa and one could envision, how much recognition potential might these proteins bear.

The second experimental observation relates to the interaction of serpins with tissue-associated immune cells that produce specific cathepsins [8,9]. Previously, we hypothesized serpins produced in the liver may define the majority of biological interactions in an organism. Serpins can protect tissues, organs, and even tumors from the activity of cathepsins. Cathepsins also block growth of cells, which are deplaced from their organ or tissue they are derived from. This phenomenon is known as homing effect (“organotrophy”). The biosynthesis of liver serpins is possibly linked to the protective effect, which is observable after liver transplantation. It is known that the rejection reactions of a host against a transplant is much decreased after liver transplantation [12]. The tolerance mechanisms of the liver may be comparable to the one of *Schistosoma japonicum*, which parasitizes blood vessels and tissues of hosts. Interestingly, these organisms have serpins which are highly homologuous to mammalian serpins. The mechanism how these serpins protect the parasite from the immune system of the host has been reported [13].

3. IS IT RELAPSE OR REGENERATION?

Early works of Babaevoj have shown that the removal of pair organs in rats also leads to an increase of the mitotic activity of cells in the residual organs. Remarkably, the time kinetics of increased mitotic activity is similar to the one observed after tumor removal [14]. Recent observations in cosmetic surgery confirm the existence of a quantitative regulatory system for tissue growth. Lipomatosis has been described after liposuction. After removal of fatty cells, the residual fat cells start growing in the esophagus, the trachea, in the kidney capsule *etc*. [15-19]. These fatty cells may bear malignant properties (relapse and metastasis) or physiological property (regeneration). As pointed out by Gorelik, there is not only a regulation of the tumor cell number, but also of the quantities of microorganisms and parasites during chronic diseases [20]. Usually, chronic diseases are hard to treat once they relapsed. This may be explained by the following mechanism: The organism cannot provide sterility of integuments and mucous. This may facilitate that certain microorganisms colonize certain sites of mucosa and tissues that are otherwise populated by other microflora. At the same time, serpins may inhibit the settlement of these microorganisms in other host tissues. Hence, the homing effect may not only be operative for the organism’s own cells but also for microorganisms during chronic infections. A similar mechanism may be valid for the phenomenon of microchimerism where a donor-specific tolerance can be observed against transplants [21,22]. In this sense, tumor-specific serum factors represent a special case of regulatory homeostasis of tissues and organs of an organism.

4. CONCLUSION

In conclusion, the phenomenon of metabolic regulation by specific proteases and anti-proteases (serpins) is well known and there are many examples in the literature, *e.g*. embryogenesis, coagulation, fibrinolysis, kinin/kallikrein system and others. In the present review, we hypothesize that this phenomenon

of regulation is of more general relevance than it was presumed earlier. Serpins are phyllogenetically ancient and highly conserved proteins in the evolution of life, which already appear in metaphytes. They may have served as immune system-like protection systems maintaining homeostasis of cells and organisms. This may apply not only for microorganisms and higher organisms such as mammals, but also for infectious diseases and cancer development. There is already a solid body of evidence in the literature reported on the validity of this hypothesis. Previous work conducted by our group and others may stimulate research on this fascinating topic to substantiate this concept of tumor growth in the years to come.

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