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The Role of Stroma in Tumour-Host Co-Existence: Some Perspectives in Stroma-Targeted Therapy of Cancer

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Abstract

Cancer grows at the expense of the host as a parasite or superparasite following the second law of thermodynamics (conservation of energy). When the cancer cell progresses via replication to the special state called "spheroid", a new phase begins with its intimiate interaction and development of responses from the stroma which together assist in the formation of a full blown cancer. Among the processes involved are the development of blood vessels and lymphatic channels which are essential for maintenance and further growth of the cancer mass. In this way the condition of "parasitism" is completed with simultaneous suppression of the immune response of the host to the histoincompatability of the tumor mass. Stroma/parenchyma promotes cancer invasion by feeding cancer cells and inducing immune tolerance. The dynamic changes in composition of stroma and biological consequences as feeder of cancer cells and immune tolerance can give a perspective for rational drug design in anti-stromal therapy. There are differences between normal and cancer cells at subcellular level such as compartmentalzation and structure of cytoskeleton and energy distribution (that is low generally, but locally high in normal cells). In cancer cannibalism of normal cells, the growing cancer mass is a factor for progression and invasion.

Cancer cells have been shown to kill normal cells and the products of cell death used for progression of growth of the cancer cell. Serum and growth factors produced by tumor stroma also provide the needed nutrients and conditions for further tumor growth. Cancer cannot feed off other cancer cells and therefore grow poorly. Probably, although not yet proven, the inability of cancer to "parasitise" other cancer cell types is probably due to some kind of competition or interference. The tumor is in charge of its own development due to its induction proteinases, lipid mobilization factors and angiogenetic factors as well as its ability to negate immune responses of the host response to what is in essence a foreign body.

In our review co-existence of normal and cancer cells in tumor with the growth promoting factors, and the immune tolerance mediating factors produced in the stromal and cancer cells/tissues will be discussed with perspective of stroma targeted therapy.

Keywords: Development of a solid cancer; 2nd law of thermodynamics; Parasitic interaction between cancer and normal cells; Stroma cells; Interaction between stroma and cancer cells and immune responses; Therapeutic targets; existing compounds; Proteases; Immune regulators; Vascular and endothelial growth factors; Inhibition of signals between cancer and normal cells of surrounding and mestatic sites

Introduction

The clinical significance of cell cannibalism is well defined and described in a large number of publications [1,2]. The direction of process of cancer developmemt is defined as the tumor invades the normal tissue which never occurs in the reverse direction. This suggests that the cancer cell strives to achieve the lowest energy level possible. Therefore the first law of the development of a full blown cancer can be considered as the 2nd Thermodynamic principle [3] that explains, describes and drives the invading cancer into normal surrouding tissue.

From the normal living state, under particular conditions such as hypoxia, where ATP synthesis is decreased resulting in a switch to glycolytic pathways, cancer cells are selected from a fraction of the population [4]. Energetically, in the presence of electron transfer, by using high energy from respiration, the proliferating state is more stable than resting cells where a higher degree of protein stabilization occurs such as that needed for maintainance of the cytoskeleton of the cell. It was proposed that tumor-promotion might be controlled or modulated by small electronic currents originating from reactive oxygen species and transported through the cytoskeletal microfilament network of the cancer cell [5].

Aerobic glycolysis is the main energy producing process in cancer cells [6]. Among many other aspects, recently the mitochondria have also been regarded as potential targets in the therapy of cancer. Several small molecules have been tested to restore their dysfunctional functions either by direct or indirect effects [7]. Because of poorly functioning mitochondria, the electron transfer component of the respiration cycle is inefficient; therefore, cancer cells have smaller Gibbs energy than

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healthy cells. This means, that these cancer cells exists in a metastable state [8] and are not able maintain normal cell structure. Therefore, the cytoskeleton system is collapsed and dielectric bilayers are formed as a lower grade of cellular structure with decreased electron conductivity. Consequently, to halt cancer growth, one has to evaluate the process of cancer cell development *in situ*, where the primary tumor is growing as well as that of the metastatic cell that is invading surrounding or distal tissues [9]. This affords one to suggest that the stroma is formed first during long term repeated oxidative stress, a process that is initially accompanied with inflammation due to an active immune response to the histo-incompatability antigens present on the surfce of the cancer cell. If the cancer cell evades the activity of killer T cells (Treg cells) by either secreting agents that reduce the response of the Treg cells or the immune system for whatever reason is ineffective (immunosuppresed states such as HIV/AIDS, pregnancy, transplatation therapy, etc.), the formed cancer cells have the opportunity to initiate tumor development. Because of the limited capacity of its electron transfer cycle, cancer cells are essentially starving cells that require glycolytically useful substrates. These substrates are obtained from the killing of normal cells by agents secreted by the cancer cell and the products yielded from dead normal cells "eaten" (phagocytosed) by the starving cancer cell which is digested by the cancer cells lysosomal system. This autophagic process of cannibalism keeps the cancer cell alive and thriving and is known as cytophagy, i.e., cannibalism of normal cells. This type of autophagocytosis results in a parasitic co-existence of tumor cells with normal cells and will determine the main pathway of interaction between the growing cancer tissue (tumor) and normal tissue where the cancer tissue gradually destroys normal tissues. This process obeys the scond law of thermodynamics-conservation of energy within a defined system.

The conduction of proteins and oxygen consumption are basically different in cancer and healthy tissues primarily due to the faulty mitochondrial structure. This difference bestows on the cancer cell an advantage over the normal cell that makes up the environment in which the cancer cell arose [10-12]. When cancer cells receive support from feeder fibroblasts for growth, they multiply independently from the ordered structure of tissue by forming a new agressive cell population that lives at the expense of normal tissue [8]. We have conducted a series of experiments that studied the specific coexistence between cancer and normal cells. Our aims were to analyse the various stages of tumor development with improving the opportunities of intervention for cancer therapy based upon the considerations of population biology and co-existance of normal and transformed cells or tissues. First of all, it should be noted that separate in vitro cultures which contain cancer cells and normal cells, repectively, when serum is omitted from the medium, the cancer cells survive for a longer period than do the normal cells [13]. When the culture contains a mixture of cancer cells and normal cells and serum is omitted, the cancer cells fluorish at the expense of dying normal cells [13]. These experiments clearly show that the cancer cells survive at the expense of the normal cells which when alive or dead, yield products that are ingested by the cancer cell.

As per the second law of thermodynamics, the developing cancer cell seeks a lower energy level for its stability. As this process continues, tremendous changes occur where the original status, including structure and function, can never be regained. In other words, the process leads to an irreversible state which progresses to lower energy levels. This lower energy state means that whereas the normal cell requires a much more stringent source of nutrients, the transformed cancer cell readily survives on cell debris, tissue fluid, fibroblasts and other connective tissue cells [14]. The limitation of avaliable nutrients does not affect the replication of the cancer cell as it does the normal cell. Whereas when insufficient nutrient is provided to the normal cell, receptors on the surface of the cell promote an inhibition of replication, the cancer cell, lacking these receptors, continues to replicate. The colonisation of surrounding tissue and organ just began from tumor spheroid state. 4) The Stroma Has a Role in the Invasion of Tumor Cells

Cancer cells invade normal tissue of an organ *via* different types of circulations, namely, the circulatory component that drains the site where the cancer cell exists and is not firmly attached to neighbouring cells or the lymphatic channels that provide similar drainage [15,16]. In the case of the circulatory system, the cancer cell may be taken to distal parts of the host's body. In the case of the lymphatic system, the cancer cell will reach the first lymphoid nodules where it is trapped, but continues to replicate. Therefore, different types of circulations specifically mediate the first steps of spreading and invasion into the surrounding tissues. The development of tumor stroma and the role of stress in tumor progression have been described in detail by several authors. There is a plethora of evidence that the tumor stroma differs significantly from that of the respective normal tissue. This is a very complex microenvironment, composed of connective tissue cells, blood vessels, lymphatics, nerves, smooth muscle elements, fat, lymphoid and macrophagic elements embedded in the extracellular matrix niche [17]. It is well established that there are dynamic and mutual interactions between the tumoral cells (e.g. tumor parenchyma) and the stroma, and recently, this concept provides the basis for novel treatment strategies by considering the opportunities given by the second law of thermodynamics [18]. The main components of tumor stroma are cancer cells, fibroblasts and immune cells. The biological role of stroma/parenchyma is feeding the cancer cells building a micro-environment for tumor and an interface between host healthy tissues and tumorous tissues [17].

The structure of the Extracelluar Matrix (ECM) or tumorous parenchyma may determine the degree of resistance the moving cell encounters. The malignant cells secreting various soluble factors that may remodel the ECM through collagen crosslinking, and tissue rigidity can potentially facilitate the directed cell migration. Tumoral chemotactic factors may trigger the monocyte → Tumor Associated Macrophage (TAM) transformation, and these cells are regarded as the major stromal cells responsible for the migration, invasion and metastasis formation. These cells are usually accumulated in the hypoxic areas of the cancer, and produce several pro-angiogenic factors [19]. In addition to TAM production, factors released from the tumor cells (interleukins, growth factors) stimulate TAMs to secrete Matrix Metalloproteases (MMPs) and other ECM degrading proteases which further enhance the invasiveness of the cancer cells [20,21]. Cancer cells can change their stroma by cell to cell contacts during tumor growth and are capable of modifying the invasiveness and metastasis formation of the tumor.

Another important cell population in the tumor stroma is the Cancer Associated Fibroblast (CAF) that is generated by transforming growth factor β1 (TGF-β1). They can produce a variety of cytokines, growth factors and ECM proteins in a paracrine manner that further alter the microenvironment of the cancer. CAFs, however, have a Janus-face, because they may promote tumor growth by enhancing angiogenesis and activating the endothelial cells, but they can also exert a tumor-suppressing effect [22]. Based on parenchyma protein production, the cancer associated fibroblast can determine the biophysical properties of the matrix favoring again the spreading of the cancer cell [23]. The main components and possible targets for intervention localised at the

border (interfase) between "tumor –normal tissue" [24] are: 1. glycolysis [6], 2. lipid mobilization factor [25] and 3. proteolysis inducing factor at the microenvironment of the tumor-host interface [26].

The Stromal Cells as Possible Targets of Therapy

The stroma and parenchyma containing the various cells and ECM can be considered as a new therapeutic target having multiple sub-targets essential for tumor growth and the immunosuppressive property of the cancer cell. Several inhibitors of matrix metalloproteases needed for cancer growth, have been shown to inhibit tumor cell growth [20,21]. When therapeutic approach co-targets parenchymal, stromal cells, the ECM and cytokine elements, a good antitumor effect would be achieved. Experimental conditions provide the opportunity to exploit simultaneously more target interaction than targeting only a single cell or a single compartment interaction [27].

When the cancer cell is formed and the spheroid state develops gradually, the transformed cells will attract feeder cells, forming a new tissue structure, in which the polarity and place of cells are disturbed. Therefore, the tissue is disordered. The newly formed disordered tissue contains the tumor cells supported by the attracted stromal cells. The tumor cells are protected and fed by stroma and now have the possibility of invading the normal tissue, where tumor stroma functioning as a "Trojan horse".

The normal physiological activities of cells in the region of disordered tissue or an entire organ consisting of disordered tissue, will present gradients of electric potential that differ from normal ordered tissue. In a conventional sense, the movement of fluids that transport ions and other necessary materials to reaction sites in the disordered tissue, severely compromise colonization of normal tissue by cancer cells [9,15]. Others have demonstrated an additional pathway for energy exchange that permits reactions among distantly situated regions of the colonized tissue [28]. The living cells at the periphery of a tumor act as a "semipermeable" sieve or barrier between the surroundings and central necrosis in the tumor. This property may at first inspection not be quite obvious. The tumors consisting of cancer cells and stromal cells make the tissue firmer and denser than the surrounding normal tissues. This general firmness and greater density of the tumor serves to identify palpable cancers rather readily (breast and prostatic cancers are good examples). The sieve functions of various interstitial channels in tumors are increased compared to the surrounding normal tissue [15].

The development of stroma begins and is subsequently followed by invasion. Hence, the network of interstitial channels in a tumor potentially constitutes a relative barrier to interstitial flow, i.e., a "tumor barrier". On both sides of the tumor barrier, non-permeable bodies may be found. Many of these biological units may then be too large to pass through the intercellular spaces of an organized tissue. In this sense the intercellular sieve of a tumor acts as a barrier where material can be adsorbed or trapped by diffusion and closed electric transports [16]. What is the relationship of the electron deficiencies in cancer that result from less than adequate mitochondrial function? Various charge transfer cycles (CTC) of primary, secondary or tertiary nature exist in biological systems [28]. The electron deficiency is not a generalized deficiency, but a very specific one where one CTC cycle is not operating or is inactive [28]. Can a deficiency of one or more CTC cycles be exploited for inhibiting tumor growth? The answer is definitely no! They are responsible for tumor formation.

In general, we think that the direction of tumor growth and invasion of surrounding normal tissues is due to mechanism of auto-phagocytosis or cannibalism (self- and xeno-cannibalisms), defined as a superparasitism, is based on the 2nd law of thermodynamics [13,18,29]. Consequently, the application of well defined external force fields- may reverse the direction of tumor growth by the modification of the direction of some particular entropy flow therefore serving to modify parasitic interaction. Exposure of the "tumor-normal tissue area" to a physical force such as pulsed application of square wave electric potential, or, chemical (e.g. specific apoptosis inducers) may yield the desired modification of tumor growth. Blocking essential local energy producing mechanisms by the inhibition of anaerobic glycolysis, inhibition of the beta-oxidation of fatty acids as growth stimulators, blocking the alternative pathway of respiration branches by the application of SHAM (salicyl-hydroxamic acid) or, by biological intervention such as immunomodulation, can be promising ways to combat cancer.

Perspectives in immunology for stroma targeted therapy

The target of tumor killing activity of a low dose alkylating agent- an anticancer drug- was dissociated from its immunomodulating activity by treating mice bearing a tumor resistant to certain alkylating drugs. Induction of specific anti-tumor response by a low dose of alkylating drugs was due to expression of "latent anti-tumor" capability [30]. This fits the conception that "suppressed concomitant immunity" occurring in tumor-bearing animals can be activated. The immunomodulating activity of alkylating drugs was related to enhancement of T-cell functions by impairment of suppressor T-cell activity, enhancement of effector T-cell activity and increased production of cytokines at the tumor site. A low dose of anticancer agent had an immunomodulating effect in human cancer such as reduction of ConA-induced suppressor cell activity in melanoma, some improvement in addition to use of melanoma vaccine, and potentiation of DTH in cancer patients. The immunomodulating effect of alkylating drugs suggests that their use might be beneficial not only for killing tumor cells but also for promoting specific anti-tumor immune response [30].

The need to modify xenogenization of tumor cells *in vivo* to make the cancer cell more immunogenic and to raise an efficient protective immune response was studied in experimental models by the use of xenogenized human tumor cells for immunotherapy by using chemically and viral modified tumor cells [31]. Modified tumor cells were found partially effective as immunomodulating agents. Moreover, the mitogenic effects of agents on alveolar fibroblasts suggest a role for in fibrogenesis in support of cancer stroma, inasmuch as the supportive component of tumor stroma was reduced [32].

Some cancer vaccines that have been used in clinical trials which have resulted in partial beneficial therapeutic effects have not provided a full solution for rational use of thymic humoral factor as immunotherapy against cancer [33]. The use of cell free mediators for cancer immunotherapy in clinical trials suggests that much remains to be done in order to assure effective and reproducible therapeutic effectiveness of immunotherapy protocols for routine use in the treatment of human cancers [34]. Epstein Barr Virus (EBV) induces production of a suppressor factor in the supernatant of B-cell cultures [35]. BCG induces in the patient immune processes that target the early stages of a urinary bladder cancer. These findings above, collectively suggest that both T and B cells play important roles in the immune regulation and immune suppression by EBV [36].

Human melanoma cells secrete a factor that inhibits phytohemagglutinin induced T-cell proliferation and lipopolysaccharide induced B-cell proliferation. It is supposed that these factors have a role

in protecting the melanoma tumor from attack by the immune system and reduce the antitumor responses of the host [37]. At any rate, antigen activated human macrophages have therapeutic activity against human tumor cells growing in mice [38]. The regulatory role of inflammatory mediators and their relationships with eicosanoids is a network that controls the expression of antitumor activity of the macrophages in cell to cell contact, and, because production of these anti-tumor factors can be shown in the medium containing the tumor and activated macrophage, these soluble factors as antitumor immunomodulators have potential for immunotherapy of human malignancies [39].

The stromal cells may regulate local immune responses by interacting cancer cells with the tumor-infiltrating T lymphocytes [40]. In addition, immune competent cells can mediate the cancer to accommodate, to adapt to the host and to avoid attack by host immune system. Stromal cells affect the immune response; create immune tolerance and barriers that avoid host immunity to the cancer cell. The tumor tolerizing mechanisms result in the inability of T-cells to destroy the tumor cells [41]. Suppression of T-cell activity is an important contributing factor by down regulation of MHC molecules, resulting in a decreased expression of HLA class I antigens [42], and the altered HLA class I phenotypes in human tumors is responsible for the immune suppression in cancer. Interestingly, the tumor cells do not only inhibit directly immune function, they kill infiltrating antigen specific T-lymphocytes [43,44]. However, these studies cannot be extrapolated to *in vivo* effects [45,31-38].

The tumor-shed soluble MHC class I homologues MICA and B are often produced by epithelial tumors resulting in the reduced responsiveness of tumor specific natural killer T cells [43,44]. In addition the tumor cells express T- cell stimulating antigens that are tolerated by the T-cells leading to insufficient antigen density [41,45,46].

Due to immune deficiencies in the host and immune tolerance of cancer cells, the tumor develops as a stealth object in the body by several mechanisms such as a tumor virus blocking the MHC1 antigen transport to the surface of the cells [47]. Consequently the cytotoxic immune response cannot eliminate the tumor cells. The production of a great variety of immunosuppressive factors involves complex mechanisms that contribute to the stealth behavior of cancer in the organism. Tumor gradually grows and invades the healthy tissues without early presentation of symptoms. Due to immunosuppression induced by human tumors, the tumor escapes immune surveillance and continues to grow [48].

Cancer cannibalism is an important mechanism of malignancy responsible for immunotolerance or resistance when metastatic tumor use cannibalism at low nutrient supply, The cannibal cells feed on sibling tumor cells and other cells under acidic environment that allow activation of lytic enzymes such as cathepsin B, and other factors as caveolin and actin-linker molecule ezrin. Various steps of cannibalisms may be explored and exploited for anti-stromal therapy [3,17,20,21,49]. From thermodynamic aspects the toxic effects of entropy flow from tumor to normal tissue plays an important role in the superparasitism, in the competitive exclusion between tumor and normal host cells where the possible targets are the proteolysis, extracellular matrix, various signal pathways, VEGF etc. [3,50]. Tumor gradually grows and invades the healthy tissues without early presentation of symptoms. Due to immunosuppression induced by human tumors, the tumor escapes immune surveillance and continues to grow [48].

As a by-product of oxidative phosphorylation Reactive Oxygen Species (ROS) are formed that may contribute to tumor initiation or progression [51]. Apart from damaging effects on nuclear and mitochondrial DNA, they are able to directly activate cell signaling such as MAP kinase, or phosphoinositide 3-kinase pathways [52], and they are implicated in the myc-induced tumorigenesis [53]. The hypoxia-inducible factor (HIF) seems to be a particularly important factor in the ROS-mediated tumorigenesis. HIF is known to mediate the upregulation of glycolytic genes and a global shift in cellular metabolism toward glycolysis, and the ROS stabilize and activate this factor under hypoxic conditions [54]. Therefore it is not surprising the targeting of mitochondrial ROS in cancer therapy is a novel approach. The tempting idea that large doses of antioxidants might interfere with the tumor development or progression proved to be inconsistent and controversial in human studies, still there are data about the ROS-mediated cell killing by different cytostatic drugs [55], or there is ongoing research to find selective anticancer compounds [56]. Similarly, targeted inhibition of HIF is also under investigation [57].

The redox-status in cancer stem cells is hardly understood. These cells are believed to be relatively radio-resistant and drug resistant because of low ROS-level and high concentration of scavenger molecules. Redox-modulating strategies could serve again new therapeutic approaches to overcome drug resistance. However, breaking their redox-adaptation has a Janus-face [58]. Nevertheless, some promising results are available indicating the selective killing effect of a thiol-depleting compound on leukemic stem cells without significant toxicity of normal hemopoetic cells [59].

Apparently the stroma has a key role in tumor development, consequently can be considered as possible target of therapy. There are opportunities in at least two different areas of therapy: a. focused on tumor stroma the modification cancer cell adhesion, proteolysis, extracellular matrix and various signal pathways as was suggested by Liotta et al. in 2002 [17]. Other opportunities are related to thermodynamics or physics. We had quantitively demonstrated that entropy production of rates of cancer cells is always higher than that of normal cells Molnar et al [60].When an electric field is applied to cells the entropy production rate of normal cells may exceed that of cancer cells [59]. The thermodynamical approaches as confirmed by calculations of the effects of external energy of electric field as the application of physical on the growth inhibition of cancer cells was a promising intervention as suggested by Luo et al. [29].

The electric effect of field was demonstrated recently *in vivo* where high electric fields induced the ablation of cancer in various metastatic tumors in mice and in clinical trials due to electric ablation in changes occurred in the tumor microenvironment. The induction of antitumor immunity was also involved in the complex effects of the pulsed electric forces [61].

Conclusion

Therapeutic opportunities that exploit various facets of cancer development, invasion, and the role that the stroma and its distinct composition of cell types play [17], are briefly summarized in Table 1. The *in situ* ablation of solid tumors by electric forces and effects on the tumor microenvironment and antitumor immunity has been discussed by others and suggestions for therapy well presented [61].

Due to the large number of distinct cancer cell types and subsequent departure from their appearance, functions, biochemical properties and energy and nutritional needs when they metastasize to different parts of the body that exhibit different stromal conditions, it is highly doubtful that any single form of therapy that specifically aims at one target, will

Target	Example agent	Comments
Adhesion	Vitaxin (anti-acfl3-mAb)	Cytostasis in patients, antitumor and anti-angiogenic in animal models
Proteolysis	Matrix metalloproteinase inhibitors	Cytostatic in patients Stromal fibrosis
Extracellular matrix	Pirfenidone	Suppresses stromal remodelling
Signal pathways	Squalamine Anti-EGFR mAb (C225) CAI (nonvoltage-gated Ca^{2+}-uptake inhibitor)	Selective to endothelial cells Active in animal models Active in combinations *in vitro*

Table 1: Examples for therapeutics targeting stroma-tumor interactions [17].

be effective against many cancer cell types. These differences may not be as limiting as one may think. Rather they may present the ability to selectively target the sensitive target of a distinct cancer cell type. This in turn may result in far less damage to normal cells as is the case with the "shot-gun approaches" of chemotherapy and radiotherapy in use today.

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