

The Tumor Microenvironment: The Making of a Paradigm

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Abstract

What has been will be again, what has been done will be done again; there is nothing new under the sun

(Ecclesiastes 1:9)

Stephen Paget was the conceptual father of the role played by the Tumor Microenvironment (TME) in tumor progression. The focus of this essay is the developmental phase of the post Paget TME research. Attempts will be made to highlight some of the pioneering work of scientists from the late sixties through the eighties of last century who laid the foundations for the contemporary scientific achievements of TME research but whose ground breaking studies are rarely cited. This review should serve as a small tribute to their great work.

Keyword Tumor microenvironment

The tumor microenvironment (TME) is a pivotal factor in tumorigenesis and especially in tumor progression and the pathogenesis of cancer is largely dependent on its interactions with microenvironmental components. This paradigm should be clear to every cancer researcher, as it is for the participants of the “5th International Conference on Tumor Microenvironment: Progression, Therapy & Prevention”.

This presentation attempts to highlight certain key events of the developmental phase of the “tumor microenvironment” concept which lead to the contemporary achieve-

ments of this research area. The essay which is not intended to serve as a comprehensive review will conclude with a biased view as to challenges facing TME researchers.

Stephen Paget laid the foundations of the TME research area by formulating the seed and soil theory. Paget’s concept lay dormant for many years. Only in the mid seventies of the 20th century and onwards did a relatively small group of people revisit Paget’s ideas [1–9]. Auerbach [10], for example, cites Paget: “The best work in the pathology of cancer is done by those studying the nature of the seed. They are like scientific botanists; and he who turns over the records of cases of cancer is only a ploughman, but his observations of the properties of the soil may also be useful”. Auerbach then expresses his own views on cancer researchers who study the tumor microenvironment: “Those individuals who study the properties of the host environment should not be ignored. Not only are the observations of the ‘soil’ useful, they provide essential information without which we will not be able to understand the nature of the metastatic process”.

From Infancy to Young Adulthood

The post Paget research of the TME was initiated by two non-interacting groups of research pioneers: immunologists and scientists focusing on angiogenesis. Until the late seventies or early eighties, these two research groups performed by far the most significant TME research.

Most of the early studies on the immune microenvironment of cancer focused on the characterization and functions of cellular and humoral immune components in the tumor microenvironment [11–36] These studies established that immunocytes including T cells [23, 32], B cells

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[14, 17], NK cells [24, 31] and macrophages [19, 20, 26, 27, 29, 33, 35, 36] have the capacity to infiltrate solid tumors in humans and in animals. Other studies demonstrated that immunoglobulins (Ig) and complement components could be detected in the microenvironment of solid tumors. Tumor cells in humans, rats and mice were found to be coated with Ig [11, 12, 18, 25, 34]. This coat was composed either of anti tumor antibodies bound to the tumor cells via the antigen binding site (in an antibody-epitope interaction) [37] or of Ig (mainly IgG) bound to epithelial or mesenchymal tumor cells via Fc receptors (FcR) expressed by such tumor cells [38]. The tumor-associated FcR was a promalignancy factor [39]. Micro-environmental factors were found to regulate the expression of the FcR expressed by the tumor cells [40].

The state of the art with respect to the immune microenvironment of cancer was evaluated by leading cancer immunologists in a UICC-supported workshop on “In-Situ Expressions of Tumor Immunity” that took place in 1978 in Tel Aviv, Israel. Some of the participants of the 1978 meeting participate also in the Versailles Conference.

The proceedings of the Tel Aviv meeting were published [41]. Most of the presentations dealt with the characterization of immune components (cells and molecules) found at the sites of solid tumors and on their functional activities.

The bottom line of the workshop’s deliberations was that the immune components that localized in the TME were relatively deficient in anti tumor activities in comparison to similar components originating from systemic sites. Some tumor-localizing components, especially tumor-localizing antibodies even enhanced tumor development.

The other group of TME pioneers led by Judah Folkman focused on angiogenesis. They realized very early that tumor proliferation was dependent upon blood supply and that the interactions of tumor and endothelial cells initiated and drove this process. Angiogenic factors were identified in various types of tumors and the possibility was raised that inhibiting such factors or their interaction with endothelial cells will be of clinical benefit to cancer patients [42–59].

With the exception of research on the immune microenvironment and angiogenesis, the areas that today occupy the forefront of TME research were essentially not represented in the scientific arena until the early-mid eighties. However, from there on, the research field of the TME moved forward, expanding and enlarging its scope to new frontiers.

Among the topics that were explored in the early eighties were interactions between the extracellular matrix (ECM) and tumor cells [60–64] and between fibroblasts and tumor cells [65–67]. These and other studies published at that time indicated that tumor-ECM or tumor-fibroblast interactions may exert either anti tumor effects or the opposite, namely pro malignancy effects.

Rudolph Virchow’s paradigm that inflammation contributes to carcinogenesis and tumor progression [68] has developed into one of the major and most important aspects of the TME area. It was demonstrated that inflammatory cells (mainly macrophages) as well as proinflammatory molecules such as cytokines and chemokines whose physiologic function is to constitute a firewall against infectious agents, are causally involved in the initiation of certain types of cancer (inflammation-linked cancers) or in tumor progression of essentially all types of cancer [69, 70]. As mentioned above, several studies from the seventies of last century reported that mononuclear cells infiltrate solid tumors [19, 20, 26, 27, 29, 33, 35, 36]. It took several years to establish that such cells are heavily involved in the pro malignancy functions of cancer-linked inflammation [69–72].

However, many, if not most components of the TME may, under certain circumstances, exert anti malignancy activities whereas under different circumstances, they exert pro malignancy effects [73]. Tumor infiltrating macrophages are no exception [74–78]. The contemporary studies on tumor infiltrating macrophages tend, however, to stress their pro malignancy effects rather than the anti malignancy functions of these cells [71, 79–86].

Angiogenesis, the immune context of tumors, the interrelationships of tumor cells with fibroblasts, components of the ECM and pro-inflammatory mediators are among the cutting edge topics of contemporary TME research. It is important to realize that the pioneering studies in these areas were undertaken at a time in which cancer genetics dominated the scene.

The discoveries made in cancer genetics in the three decade period from the early seventies until the end the nineties are undoubtedly the golden era of this research domain. The prevailing and dominating concept at that time was that genetic alterations in oncogenes and tumor suppressor genes are both necessary and sufficient to initiate tumorigenesis and drive tumor progression.

What, if any was the relationship between cancer geneticists and the “individuals who study the properties of the host environment” (to use Auerbach’s words)? Obviously both groups focused on different aspects of malignancy, holding, most probably opposing views as to the relative importance of cancer genes or of the TME to the pathophysiology of cancer. There is no indication that a direct or indirect debate between those groups took place. Seemingly the concept that assigns to cancer genes the primary role in carcinogenesis was in no conflict with the concept attributing site specific metastasis to the outcome of interactions between the seed (the tumor) and the soil (the TME). None the less, armed with cutting edge and sophisticated technologies the cancer geneticists established themselves as strong and influential policy makers while the microenvironmentalists, generating “uninteresting” data

and describing “epiphenomena” were not part of the main stream of cancer research at that time.

The nineties of last century marked a change in this attitude. The contribution of the TME to cancer progression started to be recognized by an increasing number of cancer researchers.

A primary factor responsible for this development was the revolution in biomedicine brought about by the identification and functions of molecules involved in signal transduction and the elucidation of signaling pathways [87–105].

Armed with novel knowledge and technologies it was demonstrated that gene expression in tumor cells as well as in non-tumor cells residing in the TME, is regulated by microenvironmental factors [e.g., 106, 107]. Assessment of the relative contribution of microenvironmental factors versus genetic lesions to the shaping of the malignancy phenotype of tumor cells indicated that the latter are not the sole and exclusive driver of malignancy.

For example, it was demonstrated that oncogenes and a microenvironmental factor (hypoxia) synergistically modulated VEGF expression in tumor cells and impacted angiogenesis [108]. Another study, performed in my lab, showed that the microenvironment played an important role in tumorigenesis. The tumorigenicity of polyoma virus-transformed BALB/C 3T3 cells in syngeneic mice depended on the microenvironment in which these cells were grown rather than on the content of the polyoma middle T oncogene [109].

Another important factor that helped to bring TME to the fore front of cancer research was that notable scientists from other domains of cancer research joined the ranks of the tumor microenvironmentalists.

Mina Bissell, a noted developmental biologist was early in realizing that similarly to the dependence of developmental processes on the microenvironment, also tumor progression is dependent upon the microenvironment [110]. In another article Bissell’s group wrote “Several lines of evidence now support the contention that the pathogenesis of breast cancer is determined (at least in part) by the dynamic interplay between the ductal epithelial cells, the microenvironment, and the tissue structure (acini). Thus, to understand the mechanisms involved in carcinogenesis, the role of the microenvironment (ECM as well as the stromal cells) with respect to tissue structure should be considered and studied” [111]. Bissell and her colleagues concluded: “The current dominant paradigm wherein multiple genetic lesions provide both the impetus for, and the Achilles heel of, cancer might be inadequate to understand cancer as a disease process” [112].

Holding a similar view, Ruth Sager, a leader in cancer genetics wrote in one of her last articles before her untimely departure that the oncogenes and tumor suppressor genes known at that time, “affect principally

cell cycle regulation. None are known to affect invasion or metastasis”. These genes “do not begin to account for the diversity of cancer phenotypes” [113]. Sager recommended shifting the focus from DNA to RNA i.e. to expression genetics of cancer. She also advocated the “grouping of cancer genes into two classes: class I genes are mutated or deleted, whereas class II genes are not altered at the DNA level. Rather they affect the phenotype by expression changes”. Class 2 cancer genes are those controlled by the microenvironment. A similar view was expressed, 7 years later, by Vogelstein and Kinzler [114]. They indicated that the late stages of cancer are not specifically associated with abnormalities in cancer genes (i.e. oncogenes and tumor suppressor genes).

The multitude of microenvironmental factors, their enormous activity spectrum and the complexity of their intermolecular cross talk obviously requires an interactive and interdisciplinary exchange between researchers engaged in this research domain. A group of investigators thought to promote such interactions at the international level by organizing meetings dedicated exclusively to TME. The first “International Conference on Tumor Microenvironment: Progression, Therapy, Prevention” was held in Israel on the shore of the Sea of Galilee in 1995. Among the 250 participants were several who participate in the present conference. The Sea of Galilee meeting was a truly multidisciplinary event where the focal issue, the TME, was approached and discussed thoroughly by specialists from a wide spectrum of biomedical sciences.

The 1995 conference was the impetus to establish the International Cancer Microenvironment Forum (ICMF). The forum was founded by an international group of about twenty cancer researchers from ten countries. These scientists who were joined a few years later by additional scientists became the “charter member” group of ICMF.

Informal charter member meetings were held in London (1997—hosted by Frances R. Balkwill, Imperial Cancer Research Fund); Pittsburgh, (1999—hosted by Theresa L. Whiteside and Ronald B. Herberman, University of Pittsburgh Cancer Institute), San Sebastian, (2003—hosted by Fernando Vidal-Vanaclocha, Basque Country University, School of Medicine) and in Safed (2008—hosted by the Israeli Charter Members). Present in these meetings were charter members and some invited guests. These informal meetings were devoted mainly to discussions on recent results of studies connected with the TME.

One of the resolutions of the 2003 San Sebastian charter member meeting was to upgrade ICMF. The International Cancer Microenvironment Society (ICMS) was thus established. The new society thrives to constitute a significant driving force towards the development of novel, microenvironment-related cancer therapy modalities.

The second and the third “Tumor Microenvironment” conferences were held in Baden, Austria (2002) and in Prague, Czech Republic (2004). The fourth “Tumor Microenvironment” conference was held in Florence, Italy in 2007 in a joint venture with the American Association for Cancer Research. All four meetings met, in full, the intentions of the organizers to create a friendly forum that promotes a critical review of novel basic findings and of innovative clinical studies pertaining to the TME.

The scientific seeds planted in the TME field in the early seventies of the twentieth century, bore fruit which ripened about 10–15 years ago. The TME is increasingly recognized by cancer researchers as a pivotal factor in tumor progression and as a promising venue for drug discovery. Indeed many of the novel cancer therapy modalities interfere with tumor-microenvironment interactions. A point in case is drugs that inhibit signals delivered to tumor cells by microenvironmental growth factors via the corresponding receptors [115–133].

The influx of highly capable and excellent scientists from several domains of biosciences into the TME field contributed significantly to the increased popularity of this field and to its becoming an innovative and stimulating research area.

The establishment also fulfilled its share in the acceptance of the TME as an important factor in cancer development and progression.

Compelling examples for this are statements by a former Director of NCI, Dr. Andrew C. von Eschenbach. In his update from December 2, 2003, he wrote: “the cancer cell is only part of the story in cancer development. Mounting evidence now suggests that a cancer cell interacts with its local and systemic microenvironments, and each profoundly influences the behavior of the other. These tumor-host interactions permit, and even encourage, cancer progression. Two years ago, the National Cancer Institute identified the tumor microenvironment as a priority research area in an effort to expand our knowledge of the cells and factors that normally populate the microenvironment as well as to advance our understanding of how these microenvironment components interact with tumor cells”.

Additional events that increased the impact of the TME research area were:

- The launching by the National Cancer Institute, NIH, of the Tumor Microenvironment Network initiative (TMEN) with the funding of ten Programs (<http://tmen.nci.nih.gov/>).
- The introduction of topics related to cancer microenvironment to the FP7-Health-2007 program of the European Commission.
- The establishment of the TME Working Group by the American Association for Cancer Research (<http://www.aacr.org/home/scientists/working-groups-task-forces/tumor-microenvironment-working-group.aspx>).

[aacr.org/home/scientists/working-groups-task-forces/tumor-microenvironment-working-group.aspx](http://www.aacr.org/home/scientists/working-groups-task-forces/tumor-microenvironment-working-group.aspx)).

- A huge increase in the number of publications dealing with TME. Based on PubMed data, an increase of more than eight fold occurred in the 14 year period from 1995 to 2008 (Fig. 1).
- A very large number of review articles on various aspects of the TME that appeared recently. Only a small minority out of scores of such articles is cited below [73, 134–156].
- The inclusion of “Tumor Microenvironment” as a major topic in leading international conferences.
- The recent founding of the official journal of the International Cancer Microenvironment Society—“Cancer Microenvironment” (<http://www.springer.com/biomed/cancer/journal/12307>).

It is very difficult, if not impossible, to summarize, in a single article, the state of the art with respect to each of the interaction types between the tumor and its microenvironment. Indeed it was not the aim of this article to do so. None the less an attempt will be made to draw some general hallmarks characterizing most instances of tumor-microenvironment interactions.

Before doing so, it may be useful to point out the conceptual differences between Paget’s perception of the role of the microenvironment in tumor progression (Paget’s focus was on site specific metastasis) and the modern paradigm. Paget assigned to the microenvironment a role of promoter/inhibitor of tumor cell proliferation at specific secondary sites. According to his hypothesis the microenvironment at these sites either supports metastasis by supplying growth promoting factors or alternatively inhibits metastasis by growth inhibitors. On the other hand the contemporary post Paget perception assigns to the TME an

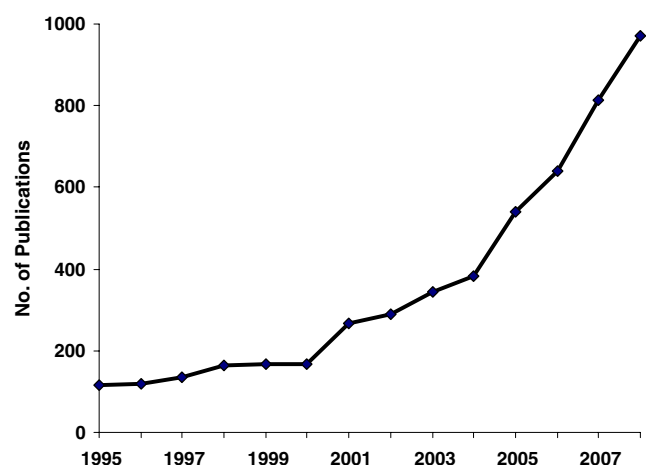


Fig. 1 Number of TME-related publications during the period: 1995–2008

inductive, adaptive and selective function: The tumor is directed into one or several possible molecular evolution pathways by signals originating in native and/or modified microenvironmental factors. Many of these pathways may lead to metastasis.

The TME may be characterized as follows:

1. The molecular composition of the TME is established jointly by tumor cells as well as by resident and infiltrating non-tumor cells.
2. Interactions of cancer cells with components of their microenvironment are crucial determinants in the decision making process determining if cancer cells will progress towards metastasis, if they will stay dormant or if they will disappear altogether.
3. Tumor-microenvironment interactions are bidirectional. Each of the interaction partners is capable of regulating gene expression in the other partner, or of exerting selective pressures on it. Each interaction partner thus shapes the phenotype of the other partner.
4. Certain tumor-microenvironment interactions may initiate and drive circular chains of tumor progression-enhancing events known as vicious cycles. In a typical vicious cycle the tumor manipulates non-tumor cells in the microenvironment and harnesses them to support its progression.
5. Certain, possibly many, microenvironmental factors play opposing roles in tumor progression by either promoting or alternatively antagonizing this process. Several variables such as the tumor type, the progression stage of the tumor, the status of certain receptors on tumor cells determine if these factors will exert either pro or anti malignancy activities.
6. Many tumor-microenvironment interactions promote tumor progression.

Destinations

Alice: Would you tell me, please, which way I ought to go from here?

The Cat: That depends a good deal on where you want to get to

Alice: I don't much care where
(*Lewis Carroll—Alice in Wonderland*)

The cancer research community, In contrast to Alice, knows where it wants to get to: It thrives to cure cancer and, hopefully prevent it.

Most of us would agree that the tumor has the capacity to shape the phenotype of non tumor cells in the microenvironment and to harness them to support its progression. Accordingly the approaches to meet the goal

of cancer cure have undergone a significant change. Cancer therapy has shifted from exclusively targeting only the tumor to targeting three components: the tumor, its accomplices and accessories in the microenvironment as well as the interactions between them.

Numerous interactions between tumor cells and the microenvironment have been identified. These interactions may either restrain tumor progression or, more often, promote it. Is any one of the pro-malignancy interactions sufficient for metastasis or do tumor cells need all (or a subgroup) of them in order to progress? Is there a hierarchy of interactions that drive tumor progression? In other words, are there more important and less important interactions with respect to metastasis formation? Are we able to identify those interactions that play the most important roles in tumor progression and should be thus, therapeutically targeted? Do different interactions integrate through intertwined signaling cascades or through shared molecules to a single interaction network?

It is up to the TME community to provide answers to these questions which are obviously of enormous importance in the design of future cancer therapy drugs. However, the immense multitude of candidate microenvironmental factors, the extreme complexity of the signaling cascades operating in the microenvironment, the intricacy of the interactive crosstalk between these cascades, and finally tumor heterogeneity, pose a formidable challenge for those of us attempting to provide solutions to these questions.

To overcome these challenges we need to provide a comprehensive overall picture of the various molecular cross-talks between tumor cells and their microenvironment leading to and driving tumor progression. One of the first steps in our attempts to comprehend the big picture of tumor progression is to realize that single molecules or single signaling pathways are just solitary components of an immense network. This realization should lead to the abandonment of reductionism (which, I am afraid, is a difficult mission under the present culture of conducting science and its funding) and to the employment of approaches used in Systems Biology [157–159].

In the interim tumor microenvironmentalists may contribute to cancer therapy by:

1. Accumulating additional data on mechanisms of tumor-microenvironment interactions
2. Finding ways to target those interactions with the highest probability of influencing tumor progression (expected are numerous opinions as to what these interactions might be...)
3. Reversing the pro-malignancy effects of the microenvironment.

These goals are achievable.

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