

# The Multiple Layers of the Tumor Environment

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Lucie Laplane<sup>1,2</sup>, Dorothée Duluc<sup>3,4</sup>, Nicolas Larmonier<sup>3,4</sup>, Thomas Pradeu<sup>3,4,\*</sup> and Andreas Bikfalvi<sup>1,4,5,\*</sup>

1. IHPST, CNRS UMR 8590, Paris
  2. Gustave Roussy, UMR 1170, Villejuif
  3. ImmunoConcept, CNRS UMR 5164
  4. University of Bordeaux
  5. LAMC-INSERM U1029
- \* Co-last authors.

## Corresponding authors:

Andreas Bikfalvi MD PhD

INSERM U1029

University Bordeaux

Allée Geoffroy St Hilaire

33 615 Pessac

France

E-mail: [andreas.bikfalvi@u-bordeaux.fr](mailto:andreas.bikfalvi@u-bordeaux.fr)

Thomas Pradeu

ImmunoConcEpT, UMR5164

CNRS & University of Bordeaux

146 rue Léo Saignat 33076 Bordeaux

France

Email: [thomas.pradeu@u-bordeaux.fr](mailto:thomas.pradeu@u-bordeaux.fr)

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## **Abstract**

The notion of tumor microenvironment (TME) has been brought to the forefront of recent scientific literature on cancer. However, there is no consensus on how to define and spatially delineate the TME. We propose that time is ripe to go beyond an all-encompassing list of the components of the TME, and to construct a multi-layered view of cancer. We distinguish six layers of environmental interactions with the tumor, and show that they are associated with distinct mechanisms, and ultimately with distinct therapeutic approaches.

## **The complex nature of the tumor environment**

Although the idea that tumors grow in and are influenced by a local environment has a long history [1], the concept of tumor microenvironment (TME) has become the focus of intense research over the last twenty years [2–7]. A multitude of papers have emphasized the importance of “context” to understand tumor development [8–10]. Moreover, the concept of TME has become clinically relevant with the development of targeted therapies against its components [11,12].

Despite a widespread use of the term, there is, however, no consensus over a definition of the TME concept. One major uncertainty, as argued by Laconi [6] is of a *temporal* nature: is the TME the pre-tumoral site that favors the development of a tumor, or the local environment induced by the tumor? [6] But there are also major issues about the *spatial* delineation of the TME, which are no less challenging, and which constitute the focus of this paper. Some researchers consider that the tumor is part of the TME, while others understand the TME as all the non-tumor components surrounding the tumor [3]. When referring to the TME, some have in mind only the elements that are in the immediate vicinity of the tumor, while others include sites that can be extremely distant, like for instance the gut with the microbiota or some remote immunological sites [1]. This heterogeneity in the concept of TME is the heritage of its rich and complex history. Indeed, three main origins of the concept of TME can be distinguished: (1) Paget’s “soil and seed” hypothesis; (2) the idea, expressed primarily by developmental biologists, that tumors develop in a structured and partly organized context (i.e., the vessels and the rest of the stroma); (3) and finally, the concept that immune cells are present within and at the close vicinity of the tumor bed and influence cancer development [2].

The consequence of this lack of consensus over the definition of the TME and the tumor environment (TE) in general is that recent reviews often take the form of long lists of

heterogeneous components or processes – from the role of the extracellular matrix to angiogenesis, immune infiltration, and to the microbiota – that are either located within the tumor or found more distantly. Drawing these lists of components, stemming from research done in different independent fields, was essential for the establishment of the TME field. Yet we propose that the scientific community working on the TME is now in need of a more precise and actionable view, namely a multi-layered view of cancer. We distinguish six layers of environmental interactions with the tumor and show that they are associated with specific location-related mechanisms, and ultimately with distinct therapeutic approaches. This leads to the recognition of different levels of the tumor environment organized as a “Russian doll” (**Figure 1**). Obviously, other hierarchical divisions of the TE than the six-layer approach we propose are possible; our main claim is not about the exact number of these layers, but rather about the crucial need to adopt a stratified approach to foster future developments in the TME field. This will improve our understanding of the TME and the TE in general and should be useful not only to scientists but also to clinicians involved in cancer research and treatment.

### **Tumor cell-to-tumor cell environment (TCTCE)**

Tumor cells evolve in a microenvironment where important interactions occur between different cancer cells. Within the tumor, the environment greatly differs between the core and the edge of the tumor. For example, the core of the tumor tends to be characterized by hypoxia, while more oxygen is available at the periphery. A “metabolic symbiosis” has been described between hypoxic and oxygenated tumor cells, indicating that each population might create a particular environment for the other. For example, the hypoxic tumor cells create a gradient of lactic acid that fuels the oxidative metabolism of oxygenated tumor cells [13].

Other types of cooperative interactions between tumor cells take place at the level of individual cells, ex., lung cancer cells can create their own niche, by producing non- or slow-proliferating

cells that secrete Wnt [14] or Notch ligands [15] supporting the growth of the more proliferative cancer cells.

Moreover, feedback loops between cancer stem cells (CSCs) and more differentiated cancer cells can play a determinant role in tumor development. For example, in chronic myeloid leukemia (CML), mature cancer cells secrete interleukin-6 (IL-6), a pro-inflammatory cytokine that induces a bias of differentiation in CML progenitors/stem cells towards the granulomonocytic lineage, leading ultimately to a feedback loop promoting CML [16].

Interactions between individual cells may also involve the construction of cellular structures such as tunneling nanotubes (TNTs). TNTs are cellular extensions that connect tumor cells to each other and could play an important role in cell-to-cell communication by transferring information (via mRNAs or proteins) from one cell to another [17]. TNTs may be organized into networks in various tumor areas and may exhibit a heterogeneous distribution.

Lastly, accumulating data suggest that genetically different sub-clones can cooperate, and thus favor tumor growth [18]. Cooperation between tumor cells may not only occur at the primary or metastatic tumor site, but also in the circulation. Indeed, recent studies highlight the existence of circulating tumor cell (CTC) clusters [19], associated with adverse outcome when compared to single CTCs, suggesting that this cooperation between cancer cells might play an important role in metastasis.

Taken together, these data indicate that the complex interactions between tumor cells already create various local environments. The interactions described above rely on various sources of heterogeneity between cancer cells, which include the classical diversity in genetic and epigenetic alterations, but also differentiation states or metabolic states. The mechanisms involved are, obviously, very local and constrained to a confined space and involves close contact or short-range interactions. These include cell-to-cell interactions that are mediated by

various processes including metabolic exchange, low-range effects of cytokines, and local structural connections between cells such as nanotubes.

We suggest referring to this first layer of environment, where these interactions take place, as the “tumor-cell to tumor-cell environment” (TCTCE). The TCTCE differs from the TME in so far as it is composed of the tumor cells themselves exclusively. The TCTCE implies cooperative interactions between tumor cells which take place, as we have seen, between individual cells or between groups of cells. The cooperative interactions are most likely reciprocal where cells have a stake on each other. In the stakeholder model [20,21] derived from evolutionary biology, the benefit conditioned by the stake must be superior to the cost. The interaction may be symmetrical (both benefit) or asymmetrical (only one benefits). Whereas single-cell cooperativity involves signaling loops between individual cells, cooperativity between groups of cells relies on a common function of groups of cells where survival advantage for both is achieved. A good example is “metabolic symbiosis”, as described above [13].

### **The niche**

The TME usually refers to the local environment in which the tumor grows. In this respect, the difference between the TME and the niche is not always clear. The notion of niche itself is used in a variety of contexts and refers to different entities.

The notion of niche is widely used in stem cell biology. Its origin can be traced back to Schofield, who introduced this notion in hematology to designate “the cellular environment which retains the stem cell”, “prevents it from maturing”, and “determines its behavior” [22][23]. Not only normal stem cells but also CSCs reside in particular niches. This is the case for hemato-oncology, where the evidence for cancer stem cells was first obtained [24], as well as for solid tumors [25]. The niche, therefore, describes the microenvironment of the CSCs specifically, as opposed to the whole tumor. It is often conceived as a “specialized

microenvironment” [26], a small unit or an anatomically distinct region within the microenvironment, in direct interaction with individual stem cells [25].

But the notion of niche is also used for cancer cells more widely. For example, in glioblastoma, a vascular tumor niche has been described and in lung metastasis the concept of metastatic and premetastatic niche has been proposed [27]. In this wider context, the notion of niche is sometimes used to refer to particular parts of the TME or to refer to the TME at a particular time point. The first case insists on the spatial aspect: the TME is composed of multiple niches. This has been proposed, for instance, in the case of glioblastoma. Hambarzumyan and Bergers [28] define niches as “communication centers in which tumor and host cell populations dynamically interact via direct cell contact or paracrine signaling cues”, and propose the presence of three types of niches in glioblastoma: a perivascular niche, a hypoxic niche, and an invasive niche. The second case insists on the temporal aspect: the niche represents the initiation site of tumor expansion. This initiation site can exist prior to tumor cell implantation, which would correspond to the concept of the premetastatic niche.

These three usages of the niche concept may overlap, but only to a certain degree. For instance, the perivascular niche is certainly found at a very early stage in tumor development whereas the hypoxic niche and invasion niche are assembled later in the life of the tumor, and these different niches can also apply to cancer stem cells. To sum up, despite this diversity in the uses of the concept of niche, they all appear as specifications of the broader notion of TME, either by focusing on the interactions of the TME with the CSCs, or by specifying various spatial or temporal small structures to which the notion of TME can broadly refer. To that extent, these various niches appear as more specific targets than the TME taken as a whole for scientific inquiry and for therapeutic interventions.

### **Confined TE, proximal TE, and peripheral TE**

In the scientific and medical literature, the notion of TME refers to a plurality of elements [1–3,7], and it is unclear whether a necessary condition for being part of the TME is to be confined to the tumor, or if elements located at more distant sites can also be part of the TME. Tumor lymphatics, for example, were not initially thought to be part of the TME in so far as they are localized at a certain distance from the tumor, and are only secondarily invaded by tumor cells. However, lymphatics were later detected inside tumors and for that reason regarded as an important part of the TME [29]. Similarly, tertiary lymphoid structures have been described in close vicinity of tumor mass, as well as within the tumor mass, in sites where priming of T cells can occur [30]. These ectopic lymphoid organs can exhibit a tumor-promoting or inhibiting role and may impact patient survival [31][32]. Cellular structures or matrix components that may impact tumor development stay outside the main tumor mass. This is the case for the tumor stroma around tumor masses, which may affect tumor development [8,33,34]. This is seen, for instance, in glioblastoma where a ring of reactive astrocytes around the tumor can be observed. Another example is pancreatic ductal adenocarcinoma (PDAC), where a very compact stroma surrounds tumor nodules, which hinders the ingrowth of vessels and the influx of immune cells into the tumor nodules [35]. Tumor-draining sentinel lymph nodes (TDNL), which have been proposed to represent an active part of the TME [36], are spatially found in a more distant location. Tumors can profoundly alter the functional characteristics of downstream lymph nodes (LNs) [37], such as priming LNs for metastatic colonization. LNs may also actively participate in tumor cell dissemination through the LN vasculature, which serves as an exit route for systemic dissemination of cancer cells [38].

Taken together, these examples illustrate the difficulty in delineating both conceptually and spatially the boundaries of the TME and the tumor environment (TE) in general. Yet the specific location and function of some elements of the TME/TE such as the stroma surrounding the

tumor or the lymph nodes located in the periphery suggest a distinction between three spatially different microenvironments, which could be referred to as the “*proximal TE*” (e.g., the surrounding stroma) and the “*peripheral TE*” (e.g., lymph nodes), in contrast to the “*confined TE*” (corresponding to the classical notion of the TME and characterized by partially permeable barriers, through which only some cells and molecules infiltrate).

Because of their specificities, these different parts of the TE might present very different challenges for tumor development. For example, from the tumor’s point of view, a key challenge in the confined TE is the local organization of the extracellular matrix, the intra-tumor vasculature, the infiltration of immune cells, and processes such as mechano-transduction [39]. The proximal TE has a double role in tumor development. On the one hand, it participates in the confinement of the tumor mass, which may contribute to tumor escape against immune responses and to therapy-resistance [40]; on the other, it maintains growth factor gradients and nutrient supply to tumor cells through vessel ingrowth or passive diffusion. For the peripheral TE, the relatively long-range communication with lymph nodes, via cellular and molecular messages, is crucial [41]. The fact that different layers are associated with different challenges and different mechanisms is of paramount importance because it delineates distinct therapeutic strategies. Indeed, approaches tailored to the reeducation of stromal cells [42] greatly differ in design and conception to those aimed at altering the mechanics or structure of the ECM [43,44], or to strategies aimed at fostering the homing at the tumor vicinity of new immune cells coming from lymph nodes [45]. Of course, these strategies are often complementary, but our point is that there exist location-dependent mechanisms in and around the tumor, and that these mechanisms can be targeted via distinct therapies.

## **Tumor organismal environment (TOE)**

Finally, one must consider the possible broader impact of the organism on tumor progression at a systemic level, suggesting the idea of an “extended microenvironment”, or “tumor macroenvironment” [46].

Perhaps the most obvious example of a long-distant effect is the priming of distant sites for secondary tumor development by soluble factors or exosomes produced by the primary tumor itself. For instance, VEGF-C derived from a primary tumor can prime lymphatic ganglions for homing of tumor cells [47]. Exosomes produced by breast cancer cells at the primary site prepare the premetastatic niche in lung tissue [48]. Along these lines, tumor-induced myeloid suppressor cells, a subpopulation of immune cells endowed with tumor-promoting activity, or tumor-primed neutrophils have been reported to promote the colonization of distant lungs by cancer cells [49,50].

Nevertheless, the role of distant parts of the organism is not restricted to metastasis; it can also concern the primary tumor. First, the hematopoietic and the immune system are connected to the tumor through the vasculature. Immune responses are induced at distance from primary tumors leading to the activation of effector immune cells, such as cytotoxic T cells, which circulate systemically through the bloodstream and migrate to the tumor sites where they exert cancer-inhibitory effects. Spitzer and colleagues have demonstrated that coordinated systemic and local immune responses are required for the success of immunotherapy [51], emphasizing thereby the importance of always seeing immune responses in the broader context of the whole organism. Second, the microbiota has been involved in the development of different tumor types (sometimes very remote from where the microbiota is localized) through its effect on the immune system. The microbiota impacts host response to various therapies, including chemotherapy and immunotherapies [12][52][53]. Such recently investigated examples further support the idea that distant elements can have a major influence on tumor development.

Moreover, cancer can have long-range effects that are well described but which feedback effect on the primary tumor or on metastases remains undetermined. For example, lung adenocarcinomas can deregulate the circadian clock of the liver and breast cancers and can impact distant tissues, in particular the hematopoietic system, by remodeling the bone marrow and increasing the immature hematopoietic compartment [54]. It has also been observed that malignant tumors can activate non-cell autonomous autophagy not only in the TME but also in distant tissues [55]. Another example is the fact that inflammatory factors induced by cancer-associated fibroblasts (CAFs) may have systemic effects and lead to decreased levels of blood myeloid and plasmacytoid dendritic cells and to a partial activation with a semi-mature phenotype and impaired immunostimulatory function [56]. Tumor cachexia can also contribute to the general weakening of the organism which impacts indirectly on tumor development and host survival.

Taken together, these data suggest a greater involvement of the organism as a whole in cancer progression than previously thought, indicating that beyond the “micro-” environment, the tumor is also exposed to a wider “organismal” environment, that we suggest to call the “tumor organismal environment” (TOE). The TOE includes, but is not limited to, what McAllister and Weinberg call the “tumor-induced systemic environment” [57], as well as the recently suggested notion of “tumor immune microenvironment” [58]. The TOE represents the full set of all the components of the organism not located in the tumor or at its vicinity but which can affect cancer development.

The wider context of the TOE raises specific challenges, such as the long-distance communication between the microbiota and tumors; this communication can be mediated by specific metabolites, by the immune system, and by the endocrine system, among other possibilities. This also creates new opportunities for therapeutic interventions, either by determining and perhaps manipulating the specific microbes that influence anti-cancer

treatments [53,59], or by acting on the communication pathways between those distant sites, including cytokines and metabolites. Clearly, a key challenge for the future will be to thoroughly investigate the role of the TOE in cancer development, its specificity in term of mechanisms, and the new opportunities that it provides for therapeutic interventions.

### Concluding Remarks

Over the last four decades, the study of the tumor microenvironment has been carried out by researchers from various disciplines [2], a fact that has contributed to explain the current loose definition and delineation of the TME in the scientific literature. We have shown herein that an undifferentiated approach to the TME, where many heterogeneous components are simply put together under a unique label, is no longer tenable. It is indeed crucial to develop a multi-layer view of the interactions between the tumor and its environments, because the challenges and mechanisms at each level are distinct, which implies that specific therapeutic strategies should be designed to target each of them. Indeed, not all tumors may harbor a complete set of layers and thus it would be important to determine which of these layers will impact tumor growth in a given tumor. For example, manipulating the microbiota through fecal transplant may not work for tumors that are immune-privileged such as PDAC. Thus, there may be tumors that command a more local intervention on the tumor environment while others require a more general approach. Of course, combinatorial strategies are important and it would be crucial for a given cancer to determine which one might work. Combination trials of anti-angiogenic therapy and immunotherapy are currently underway and we may expect that this will probably work for some tumors but not for others.

Even if we have proposed to distinguish six such layers of environmental interactions in the present paper, the exact number of layers matters much less than our more general defense of a multilevel perspective. Furthermore, the boundaries of these different levels are of course not

tight. For instance, factors that drive the TCTCE may also exhibit roles in the confined TE, such as FGFs or even VEGF which play a role in both cases [60].

One of the most pressing challenges for future research on the TME and the TE will be the dissection of the complex interactions between the different levels of tumor-environment interactions that we have distinguished (see: **Outstanding Question Box**). Not only is the “soil and seed” hypothesis constantly revisited by research that investigates how a local microenvironment can participate in the induction of distant metastatic sites [61], but it is becoming increasingly clear that overall characteristics of the host such as obesity can critically affect the local microenvironment, for example by altering the extracellular matrix [62,63]. In other words, the recognition of different levels in the TME does not mean a separation or isolation of each level, but, quite the contrary, an invitation to explore the complex and fascinating dialogue between those levels.

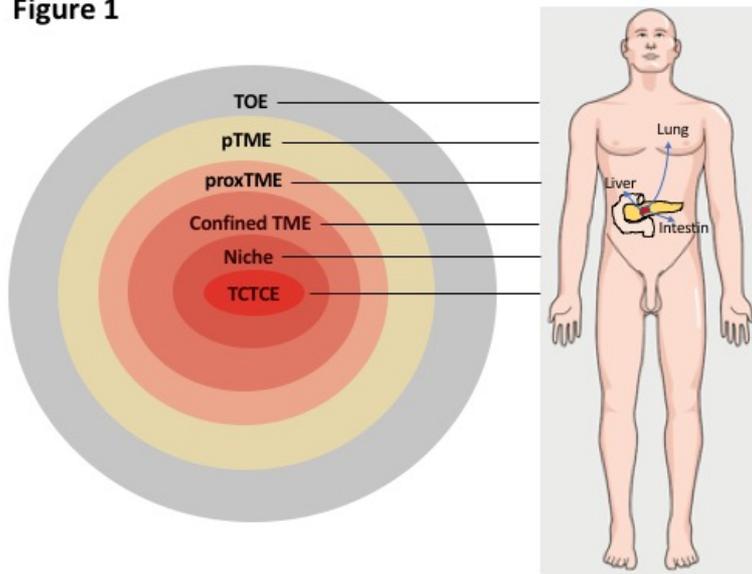
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**Figure 1**



**Figure 1: The multiple layers of the tumor environment.**

The figure on the left depicts the different layers of the tumor environment as a “Russian doll”. These different layers concern both the primary tumor and tumor metastasis.

The table on the right depicts possible therapeutic strategies related to the different TE layers. For each of the different layers, examples of its key constituents, as well as some therapeutic strategies associated with it, are listed. These therapeutic strategies are no more than possible examples, and some strategies might belong to two different layers. Yet the main message here is that distinguishing different layers in the tumor environment helps clarify the various anti-cancer treatments that can be used in the clinic. (TE, tumor environment; TCTCE, tumor-cell-to-tumor-cell environment; TOE, tumor organismal environment; TC, tumor cells; ECM, extracellular matrix; TNT, tunneling microtubes; CAFs, cancer-associated fibroblasts).