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Review

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In Vitro Modeling of Tumor–Immune System Interaction

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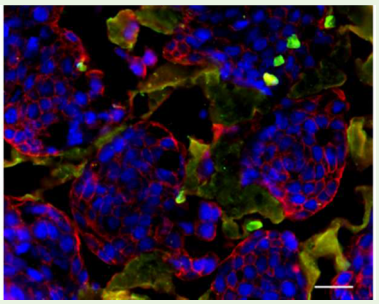
ABSTRACT: Immunotherapy has emerged during the past two decades as an innovative and successful form of cancer treatment. However, frequently, mechanisms of actions are still unclear, predictive markers are insufficiently characterized, and preclinical assays for innovative treatments are poorly reliable. In this context, the analysis of tumor/immune system interaction plays key roles, but may be unreliably mirrored by in vivo experimental models and standard bidimensional culture systems. Tridimensional cultures of tumor cells have been developed to bridge the gap between in vitro and in vivo systems. Interestingly, defined aspects of the interaction of cells from adaptive and innate immune systems and tumor cells may also be mirrored by 3D cultures. Here we review in vitro models of cancer/immune cell interaction and we propose that updated technologies might help develop innovative treatments, identify biologicals of potential clinical relevance, and select patients eligible for immunotherapy treatments.



KEYWORDS: *tumor infiltrating cells, tumor microenvironment, three-dimensional cultures, tumor engineering, tumor-immune cell interaction*

INTRODUCTION

The interaction between cancer cells and the immune system plays decisive roles in tumor outgrowth and in the control of tumor progression.[1] Indeed, tumor promoting inflammation[2] and the ability to escape immune-mediated destruction[3] do represent bona fide cancer hallmarks.[4] Studies on clinical specimens have provided a powerful validation of results emerging from experimental models and highly significant prognostic correlations have emerged from the analysis of human tumor infiltration by cells of the innate and adaptive immune system.[5] Most importantly, immunotherapies now represent routine treatments of patients with cancers of different histological origin.[6]

A variety of monoclonal antibodies (mAbs) have been routinely used for almost two decades in cancer treatment.[7] In many instances, they were developed to prevent the binding of receptors expressed by tumor cells by growth factors promoting their proliferation. However, mechanisms mediated by immune cells including phagocytosis and antibody-dependent cell cytotoxicity (ADCC) have frequently been shown to underlie their clinical effectiveness.[8] Indeed, critically depending on their affinity and isotype,[9] therapeutic mAbs may mediate target cell cytotoxicity elicited by lymphocytes or myeloid cells expressing activating Fc receptors. A main issue in mAb-mediated immunotherapy, particularly regarding innovative reagents recognizing markers expressed by immune cells, is whether it is desirable to kill target cells or rather to merely inhibit their interaction with specific ligands without killing them. In the latter case, the use of mAbs binding inhibitory Fc receptors would be recommended. Considering current uncertainties concerning the mechanism of action of several therapeutic mAbs,[10] isotype is emerging as critical for success or failure of reagents recognizing the same target molecule. On the basis of this background, reagents characterized by differential affinity and ability to bind Fc receptors expressed by effector cells are continuously being developed.[11,12] Moreover, bispecific mAbs specifically targeting defined effector functions to tumor cells are presently in advanced clinical experimentation.[13]

Most importantly, in the past decade, therapeutic mAbs recognizing immunological checkpoints have been successfully tested and utilized in clinical practice.[14] The rationale underlying their development is that they are supposed to prevent the interaction between activation markers expressed by antigen specific T cells and their ligands expressed by antigen presenting and/or tumor cells, physiologically resulting in the inhibition of adaptive T cell responses. Releasing the brakes of antitumor responses has proven effective in a variety of cancers.[15] However,

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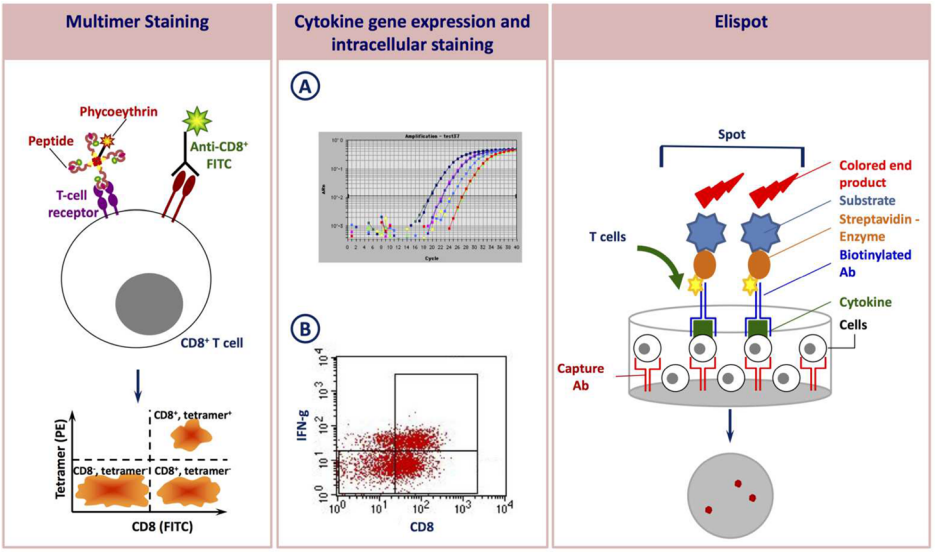

Figure 1. Currently used in vitro assays for the analysis of tumor/T lymphocyte interactions. Antitumor functions of human immune cells are currently assessed in vitro by a variety of established tests. They include the analysis of the expression of T-cell receptors recognizing tumor-specific or tumor-associated antigens (tetramer or multimer staining, left panel). Expression of cytokine genes or production of specific factors upon culture in the presence of tumor cells in standard bidimensional conditions are usually assessed by quantitative PCR (middle panel A) or by flow-cytometry upon intracellular staining (middle panel B). Elispot assays evaluate the numbers of cytokine producing cells, as detectable following culture in the presence of tumor cells or antigen presenting cells pulsed with specific peptides in standard bidimensional conditions (right panel).

mechanisms of action have not been fully clarified and markers predictive of clinical responsiveness still need to be satisfactorily identified.[10] On a similar line anti-CD47 mAbs have been used to promote tumor cell phagocytosis by macrophages.[16,17]

Adoptive cancer immunotherapies have also been developed in the past two decades.[18] They are based on the administration to patients of autologous cells following in vitro culture and expansion. Current adoptive treatments usually capitalize on the use of T cells from patients transduced with genes encoding conventional or enhanced-avidity HLA-restricted T-cell receptors recognizing tumor-associated antigens, or chimeric HLA-unrestricted antigen receptors (CAR) recognizing surface molecules highly expressed by malignant cells. While these technologies are mostly used in the treatment of hematological malignancies ongoing clinical trials also target solid malignancies.

Following these breakthroughs, a large number of innovative biologicals and procedures addressing cancer immunotherapy are being generated and tested in clinical trials and this research field is facing an unprecedented explosion of knowledge and applications, urging the development of adequate assays for preclinical assessments and for the selection of patients potentially benefiting from treatment.

MODELING HUMAN TUMOR-IMMUNE SYSTEM INTERACTIONS: THE PRESENT

Substantial knowledge underlying the development of therapeutic mAbs and innovative immunotherapy procedures has been gained from in vivo experimental animal models.[1,3,19] In vitro studies utilizing human cells have proven more problematic, not least due to difficulties inherent in the availability of sufficient numbers of freshly derived tumor or immune cells and of autologous immune/tumor cells systems. Furthermore, the generation of established tumor cell lines from clinical specimens remains a major challenge and the intrinsic heterogeneity of human cancers, in spite of a similar histological origin, must not be underestimated.

Nevertheless, conventional in vitro models have proven of paramount importance in human immunology and, in particular, in tumor immunology. ^{51}Cr release assays[20] have represented the ultimate tests for the identification of human tumor associated antigens,[21,22] and standard bidimensional cultures have allowed the expansion of tumor infiltrating lymphocytes,[23] the generation of tumor specific T cell clones,[22] and the monitoring of the effectiveness of therapeutic antitumor vaccinations.[24] Presently, flow-cytometry techniques based on the detection of cells expressing T-cell receptors recognizing antigenic peptides restricted by defined HLA determinants, for example, multimers, frequently complemented by the analysis of intracellular cytokine expression upon antigenic triggering represent routinely used technologies for the evaluation of adaptive T cell responses. These techniques are frequently accompanied by so-called Elispot assays identifying individual cells producing specific cytokines upon antigenic stimulation. Combinations of these techniques are currently included in the monitoring of antigen specific T cells responses in patients undergoing immunotherapy treatments (Figure 1).[25]

Cytotoxic activities of NK lymphocytes against malignant cells opsonized by antibody treatments are typically assessed in vitro by using tumor cell line monolayers as targets. Similar assays are also used to analyze the cytotoxic or cytostatic potential of other effector cell types expressing Fc receptors, including macrophages, dendritic cells (DCs), and neutrophils. Tumor cell proliferation or ^{51}Cr release are classically used as read-out. Phagocytosis of tumor cells by macrophages is usually tested by admixing differentially labeled effector and tumor cells in the presence or absence of biologicals of potential therapeutic relevance and using flow-cytometry to identify phagocytosed cells.[26]

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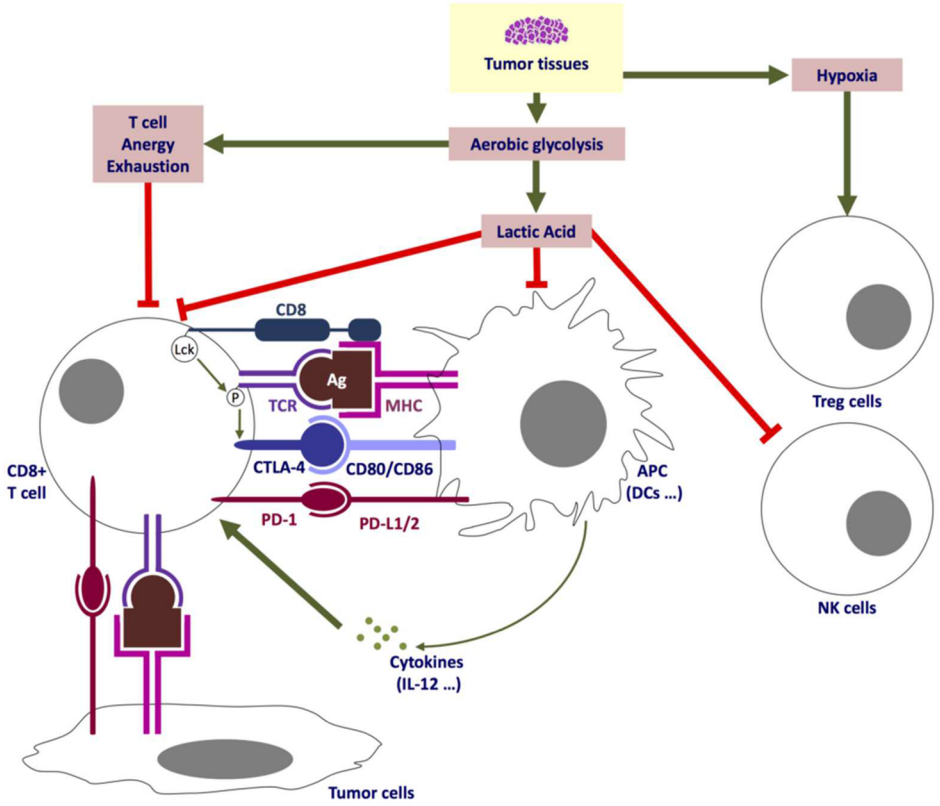

Figure 2. Metabolic alterations of the tumor microenvironment affecting tumor/immune cell interactions. The in vivo tumor microenvironment is characterized by specific metabolic features, including, among others, hypoxia and aerobic glycolysis, resulting in competition for glucose and other nutrients between tumor and immune cells and production of lactic acid. As a result, a variety of effector functions of different immune cell subpopulations are inhibited. Furthermore, functions of antigen presenting cells are also affected. At difference with standard assays, tridimensional culture systems may at least partially mirror these conditions in vitro.

WHY ARE INNOVATIVE MODELS OF TUMOR IMMUNE SYSTEM INTERACTION IMPORTANT?

In vitro data consistently indicate that, in defined assay conditions, at least T and NK lymphocytes and macrophages are able to efficiently elicit antitumor functions. Notably, however, cytotoxic tumor infiltrating T lymphocytes are frequently disfunctional in vivo,[27] as also indirectly suggested by the clinical effectiveness of immunological checkpoints targeted treatment.[28] Furthermore, immune-histochemical studies suggest that solid tumors most frequently lack detectable NK cell infiltration.[29,30] More importantly, with a few exceptions, including colorectal cancer (CRC), macrophage infiltration of solid tumors is usually associated with poor prognosis.[31]

Discrepancies between in vivo and in vitro functional profiles of immune cells have stimulated research aimed at unraveling mechanisms and conditions favoring T cell anergy and exhaustion, pro-tumor macrophage polarization, defective NK cell recruitment and, ultimately, tumor escape from immune surveillance. A variety of different cell types including alternatively activated macrophages,[32] regulatory T cells (Treg),[33] and myeloid derived suppressor cells[34] have been considered. Furthermore, immunosuppressive mechanisms at work in the tumor microenvironment have been shown include hypoxia and adenosine receptor triggering,[35,36] and expression of ligands for immunological checkpoints (see above).

Earlier reports in the past had suggested that oxygen levels may dramatically affect lymphocyte responsiveness.[37] More recently, a large number of important studies appear to indicate that hypoxia and specific metabolic conditions occurring with tumor tissues might provide a unifying background for a variety of previously observed immunosuppressive mechanisms and decisively hamper the potential effectiveness of anticancer immune responses. Indeed, hypoxia has been shown to promote immune tolerance by Treg recruitment.[38] Intriguingly, expression of PD-1 immunological checkpoint has been related to metabolic alterations occurring within tumor tissues.[39,40] A key point appears to be represented by the competition for glucose between tumor cells and T-cell receptor triggered, antigen specific T cells, both characterized by aerobic glycolysis.[41−44] Moreover pro-tumor M2 macrophage activation has also been associated with increased glycolysis,[45,46] and the development of myeloid derived suppressor cells within the tumor microenvironment has been related to hypoxia (Figure 2).[47]

While these phenomena have been extensively characterized in vivo and ex vivo, although mostly in experimental models, they also suggest the fascinating possibility of generating innovative in vitro models adding new dimensions to the analysis of the tumor microenvironment in highly controlled conditions and allowing the preclinical screening of biologicals and small molecules in conditions closer to in vivo features of the human tumor microenvironment.

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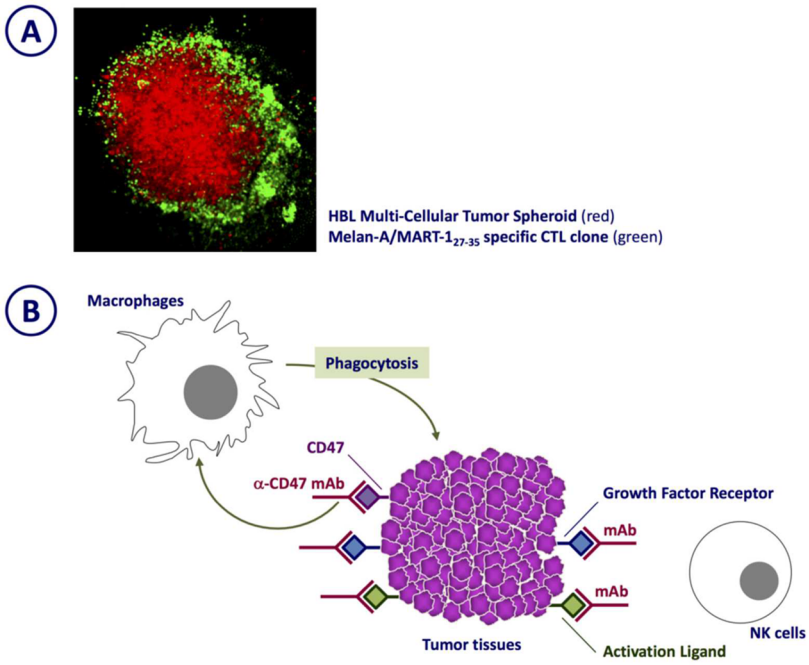

Figure 3. Tumor cell spheroids as targets of immune cell effector functions. Tumor cell spheroids generated by different procedures have been used to verify the effects of culture in tridimensional conditions on a variety of immune cell functions. T-cell clones recognizing melanoma-associated antigens have been cocultured with melanoma cells (panel A). CAR-transduced cells for adoptive treatments have similarly been tested. Functions of monocyte/macrophage lineage cells, including phagocytosis and antigen presentation have also been assessed. Moreover, antibody-dependent cell cytotoxicity mediated by NK cells has been explored using target cells cultured as spheroids (panel B).

MODELING HUMAN TUMOR–IMMUNE SYSTEM INTERACTIONS: THE THREE-DIMENSIONAL APPROACH

To address the high attrition rate in the development of innovative anticancer compounds a variety of tridimensional culture models have been developed in the past.[48] They have revealed the major role played by the architecture of cell growth in the definition of the gene expression profiles of tumor cells, their metabolic activities, and their sensitivity or resistance to drug treatment.[49−51] On the basis of these findings, innovative high throughput drug screening platforms have been generated and are currently utilized in pharmacological research. In initial studies, multicellular spheroids were obtained by preventing the adhesion of tumor cells on plastic cell culture surfaces.[52] Later, scaffolds, hanging drops, and microfluidics-based technologies were successfully developed.[53]

Control of spheroid size has allowed the generation of structures characterized by controlled levels of hypoxia and perfused bioreactors have proven to be useful to generate tissue-like structures from established human tumor cell lines.[54,55] In this context, it is also remarkable that human cancer cells endowed with tumor initiating capacity, so-called tumor initiating cells (TIC) or cancer stem cells (CSC), from tumors of different histological origin, including colon, breast, and CNS, are typically characterized by the ability of generating spheres that are able to slowly replicate with asymmetric divisions.[56,57]

Models of higher complexity are continuously being developed[58,59] aiming at including additional components of the tumor microenvironment of proven relevance in clinical course and in the development of resistance to treatment. Furthermore, physical conditions within tumor tissues and the possibility of reliably reproducing them in vitro are increasingly attracting the attention of the scientific community. In particular, microfluidics models have been generated[60] to address sensitivity to drugs and dissemination of cancer cells,[61] tumor lymphatic vessel interaction,[62] and homing of tumor cells to defined metastatic niches.[63] Intriguingly, however, the first 3D culture models had initially been developed to address immune responsiveness to solid tumor allografts.[52]

In view of this background it is surprising that only relatively few studies have addressed the effects of 3D culture of tumor cells and on their sensitivity to lymphocyte effector activities. Pioneering works suggested that tumor cells cultured in 3D were poorly targeted by cytokine activated lymphocytes[64] and that the disruption of these architecture represented an important prerequisite for a full elicitation of antitumor cytotoxicity.[65] More recently, we and others observed that T cell effector functions are severely impaired when target cells are structured in 3D architectures.[66,67]

Different mechanisms have been proposed. Dangles-Marie et al. suggested that decreased expression of heat shock protein-70 by tumor target cells might result in inefficient antigen presentation.[68] We observed that cells from established melanoma cell lines may down-regulate expression of HLA and melanoma differentiation antigens following culture in spheroids.[69] Interestingly, decreased expression of Melan-A/MART-1 differentiation antigen has also been observed in hypoxic areas of clinical melanoma specimens.[70]

On the other hand, lactic acid is produced to increasing extents in cells cultured in 3D, as compared to their 2D counterparts.[69,71] Notably, concentrations of lactic acid produced in these conditions are sufficient to significantly inhibit the elicitation of effector functions of antigen specific cytotoxic T lymphocyte (CTL) clones, thus providing an important link between typical metabolic features of tumor cells and T cell functional impairment.

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NK lymphocyte infiltration has also been studied in scaffold-free and 3D Matrigel-based models[72,73] and the impaired cytotoxic ability of natural killer (NK) cells against targets cultured in tridimensional architectures has also been reported.[74] In particular, the resistance of tumor cells to NK lymphocyte-mediated cytotoxicity in 3D glioma models has been attributed to increased HLA-E expression by tumor cells.[75] NK and Treg interaction with breast cancer cells in 3D has been shown to result in increased production of CCL4-attracting inflammatory cells of pro-tumor significance.[73] Instead, despite their potential relevance in the cancer microenvironment, there is a lack of studies investigating B-cell tumor cell interaction in 3D architectures. Most recently, models based on microfluidic technology have also been proposed to analyze tumor/lymphocyte interaction.[76]

Interestingly, recently, an advanced model based on hanging drop technology and including fibroblasts, additional key components of the tumor microenvironment has been successfully used to explore the ability of different types of immune cells to display their effector, antitumor potential,[77] as mediated by therapeutic mAbs.

A number of studies on tridimensional modeling have focused on lymphocytes. However, macrophages and other myeloid cells are also frequently infiltrating human cancers.[78] Murine and human cells of the monocyte/macrophage lineage may be polarized by cytokine treatment into M1 macrophages endowed with antitumor potential or M2 macrophages which have been shown to be rather tumor-supportive and characterized by a pro-angiogenic functional profile.[32] It is worth noting that the M1/M2 polarization notion represents a useful oversimplification of a process more realistically described as a continuum.[79] Nevertheless, the culture of monocytes and macrophages within tridimensional tumor spheroids has been shown to profoundly affect their differentiation and functional profiles.[80−82] A coculture of human and murine macrophages together with squamous cell carcinoma cells in 3D architectures, in the presence or absence of fibroblasts, has been shown to promote their polarization toward an M2 functional profile and induce metalloproteases (MMP) production, thereby favoring tumor invasiveness, as related to increased extracellular matrix degradation.[83] Similar observations were also made in experiments performed by using breast,[84,85] thyroid,[86] hepatocellular,[87] and bladder[88] cancer cell lines. In all these cases alterations of the chemokine secretome in 3D cultures including tumor cells and macrophages in the presence or absence of fibroblasts were consistently observed. NSCLC cells cultured in aggregates have been shown to preferentially attract M2 macrophages, which, in turn promote their epithelial-mesenchymal transition (EMT) and migration, as observed by using microfluidic devices.[61] In this study macrophages cultured in different conditions, potentially related to intermediate polarization stages were comparatively analyzed. Most recently, tumor cell migration in a 3D extracellular matrix was also reported to be enhanced by macrophage-secreted TNFα and TGFβ1.[89]

On the other hand, importantly, antigen presentation and differentiation capacity of DCs have been shown to be inhibited by lactic acid produced by tumor cells in 3D cultures including microfluidic models (Figure 3).[71,90] These data indicate that tridimensional models could also be advantageously used to analyze, in controlled conditions, the interactions occurring in vivo between tumor cells and cells of the monocyte/macrophage/dendritic cell lineages (Table 1).

Table 1

	3D culture system	ref
cytotoxic T lymphocyte activity assays	spheroids	52, 64, 66, 67
	engineered tumor models	76, 110
NK cytotoxicity assays	spheroids	74
monocytes/macrophage/DC	spheroids	71, 80, 81, 116
−tumor cell interaction	microfluidic devices	60, 90
therapeutic mAbs (ADCC, Bispecific Abs)	spheroids	12−14, 65, and 77
drug tests in engineered TME[a]	in vitro engineered tissue models (spheroids, microfluidics devices bioreactors)	51, 54, 55, 58, 59, 117, and 118

[a]TME: tumor microenvironment.

Interestingly, neutrophil polarization similar to functional features similar to those detected in macrophages, has also been recently reported.[82,91] However, possibly due to difficulties inherent in a granulocyte culture, the effects of incubation with tumor cells cultured in 3D on their polarization have not been addressed so far, and further research in this area is warranted.

MODELING HUMAN TUMOR−IMMUNE SYSTEM INTERACTIONS: THE BIOMATERIALS

In addition to cell composition and structural architecture, the extracellular matrix (ECM) also plays key roles in the tumor microenvironment, critically affecting cancer cell dynamics and response to treatment in vivo and in vitro.[92,93] To address these issues, a variety of biomaterials are currently being evaluated to help mimic tumor microenvironment features. While a thorough analysis of biomaterials used in 3D cultures of tumor cells[94] clearly exceeds the purposes of this review, it might be of interest to recapitulate recent advances in this area, as related to the modeling of tumor-immune system interaction.

The use of a decellularized matrix[95] from cancer specimens has been proposed.[96] However, harsh decellularization treatments might result in loss of ECM components and alterations of its ultrastructure.[95] Furthermore, ECM from human tissues are not commercially available. Notably, ECM composition may be remarkably different in cancers of similar histological origin, thus complicating standardization. For instance, in CRC, while collagen type 1 is the single most represented ECM component, laminin and fibronectin may also be present to highly different extents in different samples.[97] Useful simplifications of these complex issues might reside in the use of single most represented components[98] or commercially available ECM mixtures from experimental animals, such as Matrigel or Cultrex.[86,99] Even in these cases, however, differences from batch to batch of commercial products should not be underestimated. Agar, agarose, and hyaluronic acid have also been used for spheroid formation.[100]

In a number of reports the tumor−immune system interaction in 3D structures has been investigated in the absence of scaffolds.[66−69,72,88] In these studies spheroids might be righteously considered as building blocks of in vitro developed tumor tissues, also considering the ability of cancer cells to produce ECM components. Alternatively, collagen has been used as scaffold or to coat microfluidics devices.[61,83,89,101] Matrigel and Cultrex have been widely utilized[85,86,102] and the use of alginate[84] and synthetic materials has also been investigated.[103,104]

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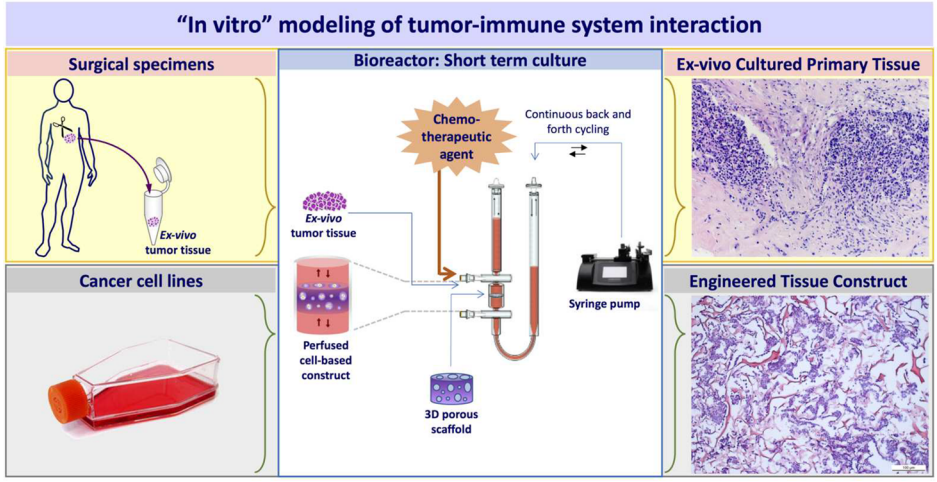

Figure 4. Innovative tridimensional models of tumor/immune cell interaction. Innovative models of tumor immune system interaction may take advantage of the use of established cell lines producing tissue-like structures upon culture in perfused bioreactors. Furthermore, the use of ex vivo cultured fragments from surgically excised cancers could also be envisaged. In either case, combinations of immune cells, biologicals and/or small molecules could be tested for their effects on malignant cells.

On the other hand, progress in the characterization of natural biomaterials and in the engineering of synthetic ones, combined with advances in the understanding of biological processes, have widely extended the range of compounds under investigation.[94] Multifunctional biomaterials targeting defined cell populations and favoring cell-to-cell interactions and crosstalk have been designed. Some of them are able to promote durable immune responses by protecting agents from degradation and providing sustained signals to host immune cells.[105−108] Therefore, biomaterials are evolving from mere structural supports into tools interacting with cells and tissues to induce and modulate biological responses.

It is tempting to speculate that 3D models of cancer-immune cell interaction will prove extremely useful for the preclinical testing of innovative biomaterials.

MODELING HUMAN TUMOR−IMMUNE SYSTEM INTERACTIONS: AN OUTLOOK

Tumor tissues include a large variety of nonmalignant cells. Their numbers may vary widely depending on the histological origin of the cancer. For instance, in melanoma, cancer cells usually account for >90% of the cells detectable within clinical specimens. In contrast, malignant cells represent a mere 10% of cells from cancer tissues in Hodgkin lymphoma. The mutual interaction between malignant and nontransformed cells is highly dynamic and critically affects both components of the tumor microenvironment.[109] In the recent past, engineered tumor tissue constructs have successfully been used to investigate the chemo-attractive potential of tumor and tumor infiltrating cells.[110]

Most importantly, the composition of the tumor microenvironment is of decisive relevance to predict the clinical course of the disease[111,112] and the response to treatment.[113] This background urges the development of techniques allowing the investigation of functional features of the human tumor microenvironment in controlled conditions. However, a number of hurdles need to be preliminarily addressed. For many human cancers, no reliable experimental model is available. Moreover, the characteristics of the immune systems of a variety of inbred murine strains poorly mirror those detectable in patients' populations.[114] On the other hand, generation of established cell lines from clinical specimens is only feasible in a limited number of human cancer types.

To obviate these difficulties the generation of patient-derived xenografts (PDTX) in immune-deficient mice has been proposed for personalized assessment of the sensitivity of tumor cells to defined chemotherapy regimens.[115] These assays are widely used in basic and translational research. However, they are characterized by a number of limitations. In vivo growth of xenografts might be difficult or require relatively long time spans, particularly for tumors of specific histological origin, such as prostate cancers. In addition, human tumor cell growth might be limited by the lack of cross-species activity of a variety of factors produced in the xenograft microenvironment. Most importantly, PDTX technologies are poorly suitable for the evaluation of biologicals and small molecules targeting tumor-immune system interaction, since human interstitial cells are rapidly replaced by murine cells in successfully growing xenografts, and human infiltrating immune cells are lost.

Ideally, innovative assays should include as many cellular components of the microenvironment of a specific cancer as possible. This represents a major challenge since primary and metastatic tumor niches may be substantially different. Furthermore, even in cancers of similar histological origin, the tumor microenvironment is highly variable and its composition might also be related to factors, for example, commensal flora in colorectal cancers poorly amenable to in vitro modeling.

To attempt to address these issues, at least in part, Majumder et al. used entire fragments of clinical specimens to predict the effectiveness of chemotherapy.[97] Limitations associated with these approaches are mainly inherent in the short timing available for testing, since a major loss of tumor viability, particularly for carcinoma tissues rapidly occurs following surgical excision. It is tempting to speculate that tumor fragments might serve as precious tools to assess the effectiveness of anticancer treatments prior to their administration to patients. A similar approach would likely require the establishment of innovative culture approaches preserving viability and functional potential of the different cell types included in the tumor microenvironment for time periods allowing the elicitation of anticancer immune effects.

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Indeed, advanced immunotherapy protocols utilizing biologicals targeting immunological checkpoints presently provide significant benefit to sizable fractions of treated patients, varying in cancers of different histological origin. However, these treatments are also characterized by a high incidence of severe adverse events. Although the identification of markers predicting responsiveness currently represents an active research area[10] relatively large numbers of patients undergo highly toxic treatments without clinical benefit. Personalized in vitro models could help to identify responsive patients prior to the initiation of therapy and novel combination approaches.

On the other hand, fragments from clinical specimens cannot be used for high throughput screening and may only be utilized to validate data emerging from less heterogeneous and more standardized models. Therefore, the establishment of more complex and realistic models of the tumor immune system interaction in vitro still represents a challenge (Figure 4).

CONCLUSIONS

It is all too obvious that in vitro models will never reproduce the enormous complexity of cancer growth in vivo. Nevertheless, they might provide the opportunity to test, in highly controlled conditions, basic science hypotheses and innovative treatments. The major advances of the past two decades have boosted an enormous interest in tumor immunobiology and immunotherapy, leading to unprecedented numbers of preclinical and clinical studies. Assessment of the effectiveness of innovative treatments will require the establishment of innovative in vitro technologies. Remarkably, the potential toxicity of these treatments will also have to be tested. Cytokine release and tumor lysis syndromes, and on target/off tumor reactivity do represent major concerns in this area and also urge the establishment of adequate in vitro models.

On the other hand, the analysis of tumor genomes and of the tumor microenvironment is challenging current tumor classification and staging criteria, usually underlying the selection of patients for standard therapeutic protocols. The emerging quest for personalized treatments might provide an additional incentive for the development of innovative culture technologies.

On the basis of this background it is easy to predict a bright future for the in vitro modeling of tumor immune-system interactions.

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