NATURE | **INSIGHT** | **REVIEW**

Influence of tumour micro-environment heterogeneity on therapeutic response

Melissa R. Junttila & Frederic J. de Sauvage

Nature **501**, 346–354 (19 September 2013) doi:10.1038/nature12626
Received 17 May 2013 Accepted 07 August 2013 Published online 18 September 2013

Abstract

Print

Tumour formation involves the co-evolution of neoplastic cells together with extracellular matrix, tumour vasculature and immune cells. Successful outgrowth of tumours and eventual metastasis is not determined solely by genetic alterations in tumour cells, but also by the fitness advantage such mutations confer in a given environment. As fitness is context dependent, evaluating tumours as complete organs, and not simply as masses of transformed epithelial cells, becomes paramount. The dynamic tumour topography varies drastically even throughout the same lesion. Heterologous cell types within tumours can actively influence therapeutic response and shape resistance.

Subject terms: Cancer

In healthy tissue, the stroma functions as the main barrier against tumorigenesis; however, the presence of transformed tumour cells initiates crucial changes that can convert this environment into one that supports cancer progression. The orchestration of these changes involves recruitment of fibroblasts, migration of immune cells, matrix remodelling and eventually development of vascular networks. How does the genetic and phenotypic variation that exists within tumours, or intratumoral heterogeneity, influence tumour growth? The identification of genetic variability within the same tumour suggests complicated events of branched evolution. Regional differences in selective pressures such as hypoxia, acidity and the presence of growth factors exist within a tumour and actively shape its development. Conceivably, distinct environmental landscapes within a given tumour select for mutations that engender survival and expansion, thereby creating tumour cell heterogeneity.

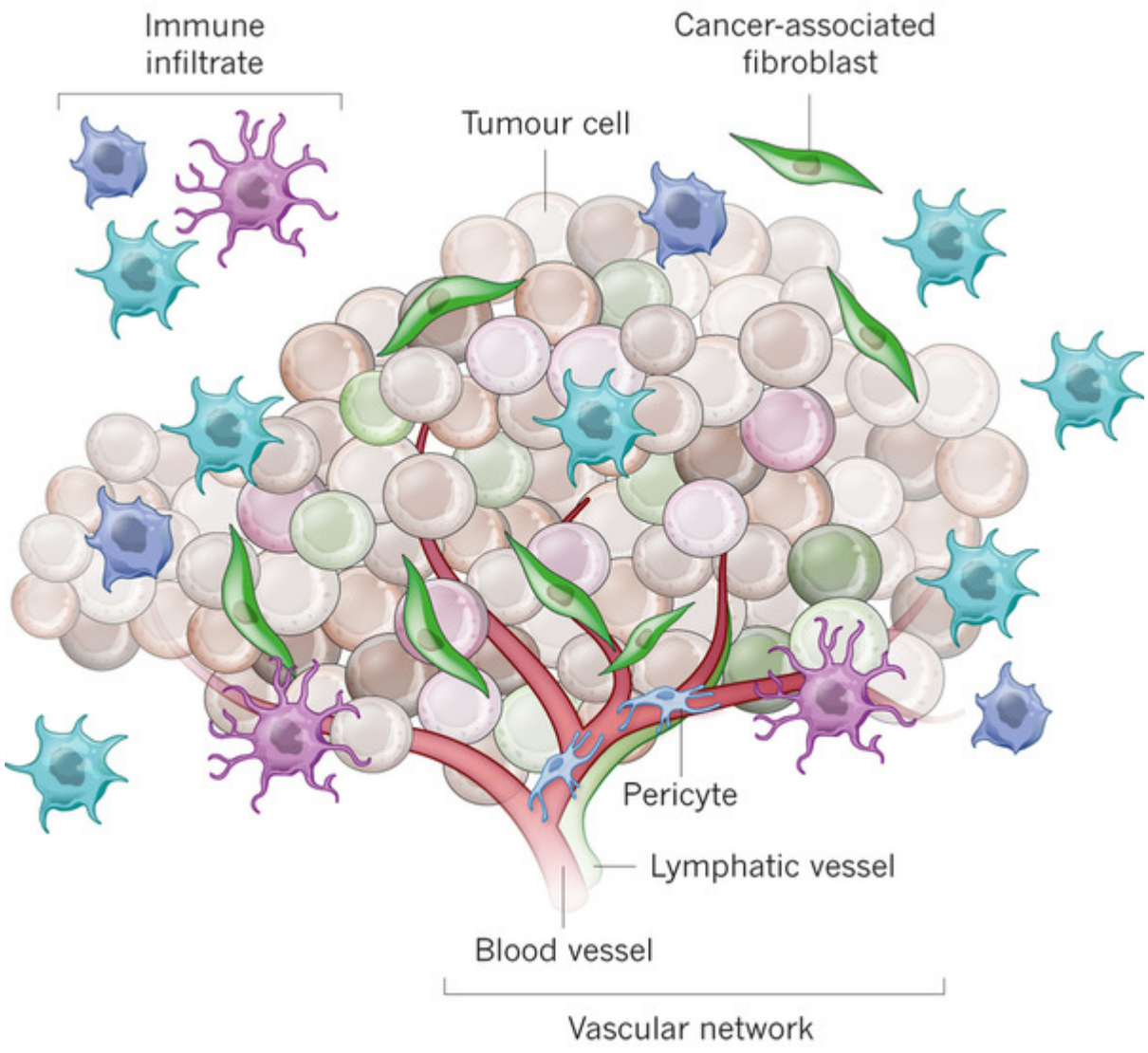
The role of tumour stroma in carcinogenesis has been reviewed extensively elsewhere[1, 2]. In this Review, we briefly define the tumour cell extrinsic compartments and discuss how they contribute to tumour heterogeneity. We then review therapeutic-resistance mechanisms that implicate stromal cell types within tumours. Last, we discuss clinical attempts to target the tumour micro-environment and the challenges that lie

ahead in order to achieve durable responses when treating cancer patients.

Origin and influence of micro-environment heterogeneity

Although the expansion of neoplastic cells generates the initial insult that instigates the creation of the tumour niche, non-transformed cell types within the milieu co-evolve with the tumour cells, so that both continuously participate in the process of tumorigenesis (Fig. 1).

Figure 1: Origins and influence of tumour heterogeneity.



Tumour formation involves the co-evolution of neoplastic cells together with extracellular matrix and vascular endothelial, stromal and immune cells. The tumour niche is a dynamic physical topography in which structural support, access to growth factors, vascular supply and immune cell interactions can vary drastically even within the same lesion. The immune infiltrate can include multiple cell types, these cell populations can have both pro- and anti-tumour functions and can vary in their activation status and their localization within the tumour. The vascular network can differ in regard to the vessel's tissue of origin, maturity (extent of pericyte coverage), interstitial pressure and functionality. Cancer-associated fibroblasts can have significant plasticity and diverge with regard to activation status, localization within the tissue, stress response and origin.

Cancer-associated fibroblasts

Fibroblasts are an abundant mesenchyme-derived cell type that maintain the structural framework in tissues. Quiescent fibroblasts differentially respond to damage, such as wounding, and become activated to support repair. Although normal fibroblasts typically suppress tumour formation[3], cancer-associated fibroblasts (CAFs) can significantly promote tumorigenesis[4]. Compared with normal tissue fibroblasts, CAFs have increased proliferation, enhanced extracellular matrix production and unique cytokine secretion (for example, stromal cell-derived factor 1, SDF1; vascular endothelial growth factor, VEGF; platelet-derived growth factor, PDGF; and hepatocyte growth factor, HGF)[5]. Other mesenchyme-derived cell types, such as adipocytes, can also contribute to tumour growth and progression.

Phenotypic and functional heterogeneity occurs in healthy fibroblasts and CAFs[6, 7]. Differences in fibroblast behaviour and response lead to extensive tissue remodelling mediated by augmented expression of proteolytic enzymes (for example, matrix metalloproteinases), deposition of extracellular matrix and pathogenic angiogenesis by liberating pro-angiogenic factors within the matrix[8]. Heterogeneity may be attributable to unique damage signals to which fibroblasts are exposed or possibly to their origin[9]. Significant cell plasticity also exists within this cell population, as both mesenchymal-to-epithelial transitions, and epithelial-to-mesenchymal transitions are known to occur, further enhancing stromal heterogeneity.

Although extensive dissection of stromal fibroblast intra- and intertumoral heterogeneity (variation between tumours) is impaired by the lack of specific markers, CAFs within tumours are clinically relevant. For example, the abundance of stromal cells correlates with poor prognosis for several forms of cancer, including breast[10] and pancreatic[11] cancer. Elevated expression of matrix metalloproteinases correlates with increased aggressiveness and poor prognosis in certain cancers[12]. A significant link between increased adipose tissue and cancer risk has also been demonstrated, although the mechanisms are still being elucidated[13].

Vasculature

The tumour vascular network is dynamic and can limit tumour growth[14]. Vascular networks are derived through formation of new vessels (angiogenesis), co-option and modification of existing vessels within tissue, or recruitment and differentiation of endothelial precursors from bone marrow (vasculogenesis), all of which contribute to vascular heterogeneity in and among tumours. Vessel formation involves degradation and reincorporation of existing vascular basement membranes that vary in a tissue-specific manner[15]. In addition, because tissue-specific vascular function and signalling are documented in normal organ homeostasis[16], this is probably also true in the tumour context. For example, it is conceivable that co-opted vessels maintain some tissue-specific characteristics, and the host tissue within which the tumour develops influences the resulting vascular network.

Uneven vascularization and differences in vascular maturity combined with a lack of drainage due to poor lymphatic vessel coverage contribute to the complex topography and variable interstitial pressure within tumours. Inadequate function of poorly organized tumour vasculatures results in areas of hypoxia and limited

nutrient supply. Distance from vascular beds creates a gradient that has been shown to be crucial for the distribution of drugs to all cells in the tumour[17]. Such variations in the vascular networks can generate distinct micro-environments within the tumour and contribute to inter- and intratumour heterogeneity, and ultimately influence clinical outcome. Microvessel density has been reported to be a significant prognostic factor for poor outcome in non-small-cell lung cancer (NSCLC)[18] and colorectal[19] and breast[20] cancer. Moreover, elevated expression of the predominant pro-angiogenic ligand VEGFA has been shown to be associated with a worse prognosis than those that have low expression of VEGFA in metastatic colorectal, lung and renal cell cancers[21].

Immune cells

The immune system collectively functions to recognize and protect tissues from infections and damage. Both the innate and adaptive immune systems have been implicated in promoting and preventing tumour growth. Although the immune system has the ability to mount anti-tumour responses, mechanisms of immune suppression can prevent this process.

Immune cell recruitment and localization in the tumour milieu vary widely in and among lesions. Heterogeneity of tumour immune contexture is influenced by various factors, including those secreted by CAF, the extent and permeability of the vasculature, and the tumour cells themselves. For instance, tumours with microsatellite instability, such as colorectal cancer subsets, may generate more neoantigens, leading to increased T-cell infiltration[22]. Similarly, the vascular bed within a tumour may strongly influence the immune contexture because endothelial cells regulate immune cell migration, as suggested by the unique endothelial transcriptional signatures of ovarian tumours that had high numbers of tumour-infiltrating lymphocytes compared with those that had low numbers[23]. Even within a given lesion, the distribution of immune cell infiltration is not uniform. When areas of cell clustering are present on the leading edge of the lesion this has a different prognostic significance to central areas of clustering, indicating the crucial nature of intratumoral localization[22].

T-cell activation involves both stimulatory and inhibitory checkpoint signals to finely tune responses to prevent excessive damage and autoimmunity. A direct means of usurping cytotoxic T-cell activation in tumours is through continuous engagement of inhibitory receptors on T-cells, such as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death 1 (PD1) by upregulation of their ligands[24]. An indirect way of preventing antitumour T-cell responses is by the generation of an immunosuppressive environment. Expansion of a myeloid-derived suppressor cell population (MDSC) — collectively referencing neutrophils, immature dendritic cells, monocytes and early myeloid progenitors — upon tumour implantation implicates tumour-initiated endocrine communication with the immune system early on[25] through tumour and CAF secretion of chemokines (for example, granulocyte–macrophage colony stimulating factor, GM-CSF; or granulocyte colony stimulating factor, G-CSF)[26, 27, 28]. Recruitment of immunosuppressive myeloid lineages to the tumour not only suppresses adaptive immunity, but also fosters angiogenesis through the secretion of VEGFA, basic fibroblast growth factor (bFGF) and transforming growth factor β (TGF-β)[29]. MDSCs also inhibit natural killer cell function and expand the immunosuppressive

regulatory T-cell population. In addition, MDSCs can directly inhibit effector T-cell expansion, activation and migration by crucially altering the environment[30]. B cells have been shown to both suppress and support T-cell function, resulting in differential effects on tumorigenesis[31]. Independent of T-cell function, B cells have also been reported to promote tumour progression by enhancing pro-tumoral inflammation[32, 33]. Mast cell recruitment has been implicated in tumorigenesis and angiogenesis[34, 35]. Tumour-associated macrophages (TAMs) can also drastically affect tumour progression depending on their polarization[36].

Immune cell subtypes have demonstrated differential prognostic value depending on the indication. For example, in melanoma, colorectal and breast cancer, a strong correlation between high T-cell occupancy and a good clinical outcome has been reported[22]. Other T-cell subsets, such as regulatory T cells, and the helper T cells T_H1 and T_H17 cells are associated with poor or good prognosis depending on the type of cancer[22, 37, 38]. In breast cancer, assessment of pro- and antitumour immunity revealed that high macrophage content relative to cytotoxic T-cell content was correlated with a worse outcome[39] than those with a low content. Interestingly, a stromal signature derived from microdissected breast tumour biopsies predicted outcome independently of tumour subtype[37]. Analysis of these micro-environmental signatures showed that angiogenesis, hypoxia and macrophage-mediated immune suppression signatures were associated with poor outcome. In addition, B-cell content has been associated with a good prognosis in ovarian[40], as well as breast[41] cancer. Together, such studies provide a strong argument for the consideration of the immune contexture as a powerful contributor to tumour development.

Resistance to therapies mediated by tumour stroma

In recent years, medical oncology has focused heavily on personalizing therapeutic approaches with the aim of identifying patient subpopulations that would benefit from specific targeted therapeutics. For example, metastatic melanoma patients who harbour *BRAF* (V600E) mutations and received treatment with the BRAF-mutant-specific inhibitor vemurafenib demonstrated marked responses[42]. As effective as these compounds are, however, resistance does ultimately develop[43].

The exploration of therapeutic resistance has largely focused on the tumour cell. Most documented resistance mechanisms involve secondary pathway mutations or bypass mechanisms within the tumour cells, such as *EGFR* (T790M)[44] mutations or MET receptor amplification[45, 46] in patients with *EGFR*-mutant NSCLC treated with EGFR inhibitors and *NRAS* mutations acquired in patients with *BRAF*-mutant melanoma treated with BRAF inhibitors[47]. However, the recent identification of mechanisms of therapeutic resistance that were conferred largely by alterations, not in the tumour cells, but in their environment, indicates the importance of understanding the tumour cell extrinsic compartments[48].

Fibroblast-mediated resistance

Early co-culture experiments demonstrated that damaged or irradiated fibroblasts could better support tumour cell growth than non-irradiated fibroblasts, suggesting that within a solid tumour, fibroblasts are not passive elements and could potentially respond and affect therapy[49, 50, 51]. In addition to the cell-autonomous mechanisms already described, recent work has demonstrated that ligand-dependent

activation of receptor tyrosine kinases can lead to resistance. Stromal derived HGF has been shown to render tumour cells resistant to BRAF inhibition in cell lines that harbour the *BRAF* (V600E) mutation[52, 53] through increased phosphorylation of its cognate receptor, MET. Moreover, abundance of HGF expression in patients significantly correlated with reduced responsiveness to drug treatment[52]. It remains to be seen whether combinatorial targeting of HGF or MET, together with an inhibitor of *BRAF* (V600E) will lead to more durable responses in patients with *BRAF* (V600E) mutation. In a similar example, elevation of pro-angiogenic platelet-derived growth factor C (PDGF-C) expression by CAFs could mediate refractoriness to anti-angiogenic therapy *in vivo*[54].

These examples illustrate the importance of assessing potential stromal mediators of inherent resistance and highlight the challenge of elucidating how therapeutic treatment could elicit resistance through unforeseen stromal changes. In a preclinical model of genotoxic injury, investigators identified secreted factors from normal human fibroblasts and showed that WNT-16b could crucially limit tumour response through paracrine signalling. Increased levels of ligand enhanced tumour cell proliferation and promoted a mesenchymal phenotype in a WNT-dependent manner. Depletion of WNT-16b specifically from fibroblasts enhanced chemotherapy response[55]. Secretion of WNT-16b from stromal fibroblasts depended on an NF-κB mediated pathway known to mediate stress and inflammatory responses. The response of the supporting stroma to treatment may show a more complicated picture in which stress-response programs in these cells may be limiting treatment efficacy by providing a protective environment for tumour cells.

Vascular-mediated resistance

It is speculated that tumour vasculature serves as a barrier to optimal drug delivery[56]. Tumour vasculature can be constrained by the dense nature of the tumour stroma, leading to compression of tumour vessels, disruption of efficient blood flow and elevation of interstitial pressure, all of which may obstruct movement within and across tumour vessels. Recent work suggests that cytoreduction of the stroma, in this case through enzymatic destruction of hyaluronan, could reduce interstitial pressure and improve vessel patency and flow[57]. The implication is that drug delivery would theoretically be improved, as indicated by the increased efficacy of standard-of-care chemotherapy when combined with hyaluronan depletion in an animal model of pancreatic cancer[57].

Normalization of leaky vascular beds within tumours through VEGFA pathway inhibition has also been proposed to transiently increase drug delivery in solid tumours[56]. However, the clinical data pertaining to this hypothesis are mixed. For example, when co-administered with anti-VEGF therapy, delivery of radiolabelled chemotherapy was slightly decreased within the tumours of NSCLC patients[58] monitored using positron emission tomography (PET). The biological consequence of these delivery changes is unclear because patients still derive benefit from combination treatments, but changing the delivery may have implications for dosing sequence — this is still under investigation.

Recent work has highlighted a direct role for endothelial cells in tumour response to therapies through secreted factors. Treatment with the chemotherapy drug doxorubicin induced thymic endothelial cell

production of the growth factors interleukin 6 (IL-6) and tissue inhibitor of metalloproteinase 1 (TIMP-1), rendering the thymus a protective reservoir for tumour cells during treatment[59]. The concept that a physical niche protects tumour cells during drug treatment has also been suggested for the perivascular space within tumours. The observation of tumour re-initiating cells along tumour vessels[60, 61] suggests these locations have a protective role. Paracrine signalling from endothelial cells within this niche has been shown to increase chemoresistance by inducing a stem-cell-like phenotype in a subset of colorectal tumour cells[62]. Similarly, hypoxic regions of tumours can harbour and support the survival of colon cancer stem cells during chemotherapy[63]. Studies such as these suggest that distinct niches within a tumour could support and instruct tumour regrowth following treatment. These insights highlight the conundrum of the unanticipated effects that therapeutic interventions can have on non-tumour cell components, which can then limit treatment efficacy.

Immune-mediated resistance

As already mentioned, characterization of immune infiltrate has generated a great deal of interest as data demonstrate that tumour immune contexture may be used to predict patient outcome. This observation suggests that the immune system is an active component of the disease and could affect clinical response and resistance.

In the context of anti-angiogenic therapy, tumours may be rendered refractory to anti-VEGF therapy by a pro-inflammatory micro-environment that includes multiple cell types such as immature myeloid cells and TAMs that secrete factors compensating for VEGF loss to support angiogenesis[64]. Depletion of MDSC expansion and recruitment that is mediated predominantly by secretion of G-CSF in anti-VEGF insensitive experimental models could rescue responsiveness to VEGF depletion, leading to decreased vessel density and tumour growth[26, 65].

Similarly, recent work has suggested that macrophage abundance may impede therapeutic responses. After radiation treatment, levels of factors that mediate macrophage trafficking were significantly increased in the serum of patients with prostate cancer[66]. In patients with node-positive breast cancer, who had undergone intense chemotherapy, those harbouring tumours with a high macrophage, high CD4, but low CD8 T-cell signature had significantly reduced recurrence-free survival[39] compared with patients who had a low macrophage, low CD4 and high CD8 signature. Experimentally, cytotoxic chemotherapy or radiation treatment increased factors that mediate macrophage trafficking and intratumoral macrophages. By preventing treatment-induced macrophage recruitment through blockade of the cognate macrophage receptor, colony stimulating factor receptor-1 responses were improved. Together, these results suggest that clinical responses to chemotherapy or radiotherapy in breast and prostate cancer may be improved by preventing treatment-induced recruitment of suppressive macrophages.

In pre- and post-treatment biopsies from patients with melanoma who received a BRAF inhibitor alone or in combination with an MEK inhibitor, there was an increase in tumour antigen expression that correlated with increased infiltration of cells that carry the CD8 antigen ($CD8^+$). This result suggests a positive contribution

of cytotoxic T cells to the therapeutic effect. Interestingly, when patients were re-biopsied at time of progression, the $CD8^+$ infiltrate had decreased concomitant with the emergence of markers of immune cell exhaustion and T-cell inhibitory ligands[67]. This example implicates adaptive immunity in both efficacy and resistance to targeted therapeutics.

Therapeutically harnessing the anti-tumour effects of adaptive immunity in patients has been clearly demonstrated through adoptive cell transfer (ACT). The aim of ACT is to boost a patient's anticancer immunity by transplanting T cells that recognize tumour-specific antigens, leading to recognition and elimination[68]. Although powerful, the responses are not always sustained. Recent work in an experimental model of melanoma suggests that inflammation resulting from the initial tumour response leads to environmental changes that induce loss of the targeted tumour antigens. The presence of tumour necrosis factor-α (TNF-α) secreted by infiltrating macrophages was identified as the key contributor to the change in target expression by tumour cells[69].

In summary, the immune system can be implicated in both inherent, as well as acquired resistance to targeted therapies. Even in cases in which the immune cells are actively driving the initial responses to targeted therapies, a wide array of immune suppressive mechanisms may eventually evolve, ultimately leading to tumour progression.

Targeting the tumour micro-environment in the clinic

An adaptive, continuous dialogue exists between tumour cells and their surroundings, mediated through direct cell contact with stromal components or through secreted signalling factors (cytokines, chemokines and growth factors). As already discussed, the tumour niche shapes responses to the selective pressure of drug treatment and may affect the emergence of resistance. Can durable responses result from multi-pronged approaches that target both the tumour cells and their cell-extrinsic support? To envisage a scenario in which targeted therapeutics for multiple compartments are successfully implemented, it is important to review what has been developed and clinically tested for targeting the tumour stroma (Table 1).

Table 1: Examples of therapies that target the tumour stroma listed by compartment

Targeting stromal fibroblasts

The impressive preclinical data and the potential broad application of matrix-metalloproteinase inhibitors for oncology has fuelled clinical development and testing of several pan-protease inhibitors, including tanomastat, marimastat and prinomastat. These agents failed to show significant benefit over the standard-of-care treatment across multiple forms of cancer[70]. Retrospectively, several explanations have been posited to explain these outcomes. Although matrix metalloproteinases are almost ubiquitously overexpressed in human tumours, it is likely that early-stage tumours are more dependent on their activity than late-stage, established tumours. Moreover, our incomplete understanding of matrix metalloproteinase biology and expression in advanced carcinomas at that time could explain some of the failures. For example, tanomastat does not target the crucial matrix metalloproteinases expressed that correlate with poor

prognosis in small-cell lung cancer[70, 71].

Targeting signalling pathways that affect tumour mesenchyme have also yielded disappointing results thus far. As is the case for the Hedgehog signalling pathway in which extensive preclinical data showed increased expression of Hedgehog ligands in many tumour types. Hedgehog ligands released by tumour cells lead to activation of the pathway in stromal fibroblasts[72], inducing the production of secreted factors that contribute to tumorigenesis by acting on tumour cells or other stromal compartments such as the vasculature. However, in contrast to tumours in which the Hedgehog pathway is mutated, the inhibitors of the protein smoothened, vismodegib and saridegib, have so far failed to show a benefit in colorectal[73], ovarian[74] or pancreatic[75, 76] cancer when combined with standard-of-care therapies. It has been postulated that Hedgehog pathway inhibition would reduce the desmoplasmic stroma within pancreatic tumours and lead to increased vessel formation, thereby facilitating drug delivery[77]. However, in preclinical models, this effect led only to very small and transient responses that did not translate in clinical trials — in which combined treatments of Hedgehog pathway inhibitors with chemotherapy have not shown clinical benefit compared with chemotherapy alone[75, 76].

Targeting vasculature

The development of molecules that target VEGFA pathways represents the most successful approach so far in targeting the tumour environment. Bevacizumab, a humanized, monoclonal anti-VEGFA antibody was the first molecule targeting this pathway to be approved by the US Food and Drug Administration (FDA)[78]. In combination with chemotherapy, bevacizumab provides a benefit to patients with advanced NSCLC[79] and metastatic colorectal cancer[80]. A benefit was also demonstrated for metastatic renal cancer[81] when the antibody was combined with interferon-α and recurrent glioblastoma multiforme (GBM)[82, 83] as monotherapy. More recently, improvements in progression-free survival were reported in patients with high-risk ovarian cancer[84] and in overall survival for patients with advanced metastatic cervical cancer[85]. The FDA has since approved several small molecule inhibitors, including sunitinib, axitinib, pazopanib, vandetanib, cabozantinib and sorafenib, that target the VEGF receptor VEGFR2 and other receptor tyrosine kinases for multiple cancers.

Although all clinical trials have demonstrated some degree of activity for bevacizumab in combination with chemotherapy, the extent varies depending on the cancer type, with only marginal activity seen in pancreatic cancer[86] for example. Although it is unclear what drives heterogeneity in sensitivity across cancer types, it is plausible that it could be due to inherent differences in vascular maturity, expression of compensating pro-angiogenic factors and differential dependency of tumour cells on oxygen and nutrients. Pancreatic tumours are noted for their hypovascularity[77], implying a lesser dependency on vascular supply than other tumour types.

Recent preclinical data have suggested that small molecule anti-angiogenic therapy could increase invasiveness and metastasis[87, 88]. Increased metastasis was confirmed with a VEGFR tyrosine kinase inhibitor (TKI) at supraclinical dose levels, but not with anti-VEGF monoclonal antibodies in multiple

autochthonous animal models[89], consistent with clinical data in many epithelial cancers, including metastatic breast, kidney, colorectal and pancreatic cancers[90, 87]. Newly evaluated clinical data from metastatic renal cell carcinoma failed to show evidence of accelerated disease or a worse outcome with the VEGFR TKI sunitinib[91]. These reports highlight the need to distinguish between different classes of therapeutic that target the same pathway and emphasize the importance of accurate preclinical modelling of clinical dosing regimens[92]. GBM may be an exception for which clinical data support the possibility that inhibiting VEGF in a subset of patients may exacerbate the invasive phenotype of their tumours[93]. Recent preclinical data have provided a context-specific mechanism in GBM, whereby VEGF functions as a negative regulator of invasive signalling pathways driven by the MET receptor that is expressed in tumour cells[94], suggesting that combination therapy with MET inhibitors may be worth exploring.

Targeting immune cells

The recent approval of several immune modulatory therapies for the treatment of cancer has reinvigorated the field of tumour immunotherapy[95]. In 2010, the FDA approved a cell-based therapy called Provenge (sipuleucel-T) for the treatment of castration-resistant prostate cancer. Although the clinical results showed no tumour regression, overall survival was increased by more than 4 months[96]. This was followed shortly thereafter by the approval of ipilimumab, a monoclonal antibody targeting the negative immune checkpoint protein CTLA-4 (ref. 97). Patients with resistant, refractory melanoma demonstrated a two-fold increase in survival when treated with ipilimumab. In a follow-up trial, ipilimumab also showed significant efficacy in naive melanoma patients together with chemotherapy, when compared with chemotherapy alone[98]. By blocking CTLA-4, ipilimumab allows for enhanced antitumour effector T-cell function and is also thought to inhibit immunosuppressive regulatory T-cell function. Based on these clinical successes, there is renewed interest in the clinical development of therapies that either block immunosuppressive mechanisms, such as PD1 and its ligand PDL1, to restore T-cell function or enhance immune function by engaging co-stimulatory receptors such as OX40 with agonist antibodies.

Other technologies that are currently in clinical development attempt to directly engage T-cell-mediated killing. Early clinical experience of blinatumomab, a bispecific antibody fragment with dual affinity for CD3 on T cells and CD19 on B cells, in patients with non-Hodgkin's lymphoma is promising because exceptionally low doses of this agent led to tumour regression[99, 100]. Such direct tumour targeting of T cells is attractive in that it provides specificity to T-cell-mediated lysis of tumour cells and may avoid most immune escape mechanisms.

Targeting components of the innate immune system is also under clinical evaluation. For example, an anti-CD40 agonist antibody has shown early clinical promise in combination with gemcitabine in patients with pancreatic cancer[101]. The mechanism underlying these responses has been attributed to macrophage infiltration, rather than enhanced antigen-presenting cell function and T-cell activity.

Therapeutic combinations

Given the growing availability of agents targeting different tumour compartments, it seems promising that

combination therapy may lead to more robust or durable responses. The fundamental question is how to combine these agents clinically. Clearly, a deeper understanding of their mechanisms of action will be required to guide rational drug combinations.

Chemotherapy may pose a challenge for combination treatment with stromal targeting strategies. Although bevacizumab is tolerated and effective when combined with standard-of-care chemotherapy regimens across multiple indications, it is uncertain whether chemotherapy and immune modulatory therapies will be combined successfully. Chemotherapy could potentially enhance efficacy of immunotherapies through multiple mechanisms, such as increased antigen production, improved antigen presentation, augmented T-cell response and trafficking[102]. However, chemotherapy can antagonize immune modulatory effects[103]. In addition to certain chemotherapies leading to neutropaenia, the type and context of cell death induced may be crucial for the success of combinations. The requirement for adaptive immunity in mediating chemotherapy-induced responses is still unclear, as divergent results have been reported using independent model systems[104, 105]. This highlights the impact of preclinical model choice on experimental outcome. Although it is unclear how researchers would predict effective combinations of therapy for certain tumours, recent clinical success of immunotherapy–chemotherapy combinations, for example ipilimumab with dacarbazine[98] for the treatment of metastatic melanoma and vaccination with docetaxel for metastatic androgen-resistant prostate cancer[106], should force the further consideration of chemotherapy and immunotherapy combinations.

The role of the endothelium in regulating immune cell trafficking suggests there is an intimacy between endothelial cell modulation and immunotherapy that is worth exploring. Strategies that increase vessel patency have been linked to increased immune cell infiltration into tumours[107, 108]. In experiments, otherwise inert tumour vaccination was found to be rendered efficacious, possibly through modulation of the endothelium allowing enhanced immune cell passage and tumour access[23, 107]. Furthermore, by using adaptive transfer techniques VEGF blockade could increase intratumoral infiltration of immune cells[109]. It has also been postulated that anti-angiogenic treatment could enhance dendritic cell function, thereby enhancing immunotherapy responses[110]. The rationale for anti-angiogenic and immunotherapeutic combinations is compelling and clinical interrogation of such combinations is ongoing.

Combinations of targeted therapies that may synergize are currently being explored. Clinical interrogation of the ipilimumab and the *BRAF*-mutant kinase inhibitor vemurafenib combination is currently being tested in metastatic melanoma. MEK pathway inhibition has been demonstrated to hinder T-cell function, despite the ability of inhibition to increase melanocyte differentiation antigen expression[67, 111]. However, this is not the case when the pathway is inhibited as a result of vemurafenib treatment, which does not affect T-cell function[112]. Interestingly, in pancreatic cancer, MEK inhibition has been linked to the reduction of MDSC populations that permits T-cell-mediated tumour targeting[27, 28].

Future challenges

Despite the success in targeting non-tumour cell compartments, significant challenges still lie ahead for

implementing stromal targeting strategies in clinical practice. These range from difficulties in assessing the composition of the stroma in human tumours to correctly modelling preclinically the vast heterogeneity observed in human tumours and correlating this heterogeneity with outcome, drug response and drug resistance.

Accurately modelling tumour stroma complexity and heterogeneity preclinically is a challenge[113] (Box 1). Not least because the complexity is poorly defined. The complexity of the tumour stroma at various stages of development and after drug treatment remains to be characterized in great detail, and this presents its own challenges. Modelling the multiple non-tumour cell types may be particularly complicated, as most preclinical techniques rely on implantation of tumour cells in foreign sites (most often under the skin, where the stromal representation may be different) or in immune compromised hosts that lack crucial immune effector cells. Looking ahead, the preclinical evaluation of combination therapies that target multiple tumour components will require the incorporation of several types of preclinical system and most likely the development of complex genetically engineered models.

Box 1: Preclinical model systems

Expand

Another obstacle in targeting the tumour stroma is developing reliable diagnostic markers that are based on a clear understanding of the determinants of responsiveness. For example, although numerous attempts have been made to identify predictive biomarkers for the effectiveness of bevacizumab, so far, no reliable and reproducible predictive biomarkers have been identified[21, 114, 115]. Intra- and intertumour heterogeneity may explain why a biomarker has remained elusive for therapies targeting the tumour stroma in general, and for anti-angiogenic therapies in particular. For instance, in metastatic NSCLC, primary lung tumours and their matched brain metastases lack correlation with respect to micro-vessel density, vascular maturity or VEGF expression[116], illustrating how tumour vasculatures vary drastically even within the same patient. These vascular differences may be reflective of the unique anatomical locations within which the lesions emerged, which is suggestive of micro-environmental influence on tumour growth, but nonetheless confounds the predictability of therapeutic response. Similarly, intra- and intertumour heterogeneity is observed with immune infiltrates, significantly complicating the evaluation of predictive biomarker identification. Additional challenges for immunotherapy lie in determining diagnostic and biomarker criteria for therapies whose targets are particularly heterogeneous or of low abundance in the tumour, as is the case for anti-CTLA-4, in which only a subset of non-tumour cells express the CTLA-4 ligand. In this case as well, despite concerted efforts, no reliable biomarkers have been identified[24]. These observations highlight the inherent difficulty in the development of predictive biomarkers for therapies that target highly heterogeneous and somewhat discrete compartments in tumours.

In light of immense tumour heterogeneity, it is unclear how and when to clinically evaluate tumour heterogeneity. As discussed, the complexity of the tumour is a result of continuous crosstalk between the tumour cells and the environment over time, adding the complication of temporal heterogeneity on top of spatial heterogeneity. Given that patients with cancer are often treated through multiple lines of therapy, serial biopsies from different sites may be required to predict response and implement therapy. This is particularly relevant for treatment-induced stromal alterations that are not observable from pretreatment biopsies. Although serial biopsies could be incorporated into routine practice as a means to inform and guide each line of treatment, as has recently been done[117], this poses a significant burden on patients.

Another challenge is how to measure clinical benefit for agents that target the tumour stroma. Patients can derive benefit from bevacizumab after disease progression[118], suggesting that the traditional definition of disease progression based on Response Evaluation Criteria in Solid Tumors (RECIST) may not be the best method to measure drug benefit when therapeutically targeting the tumour stroma. Immunotherapy strategies have unique challenges in this realm. For example, regulations of response criteria needed to be altered for this class of oncology medications[119] because patients demonstrate delayed regression or even pseudo-progression after treatment.

Finally, understanding and managing stroma-mediated resistance would have a profound affect on therapeutic strategies that target this compartment. The emergence of therapeutic resistance should theoretically be reduced if the target population is genetically stable; targeting the tumour stroma, therefore, could be advantageous because mutations have rarely been identified in the stroma[120, 121, 122]. Support for this hypothesis may be gleaned from anti-VEGFA clinical data. Following disease progression in patients on chemotherapy, bevacizumab treatment provided a benefit when given with a new chemotherapy regimen[123]. Moreover, clinical bevacizumab efficacy was evaluated when given during first-line treatment with chemotherapy and then continued with an alternative regimen at progression. The addition of bevacizumab to second-line therapy significantly improved overall and progression-free survival, demonstrating that progression in first-line therapy was not due to anti-VEGF resistance[118]. Despite the proposed advantage provided by genetic stability of the stroma, this tissue will continue to evolve during treatment — and adaptation by non-genetic means may complicate the scenario, especially given that under treatment pressure epigenetic changes within the stroma have been reported[124, 125].

Therapeutic resistance reflects active tumour evolution, and environmental mediated resistance is illustrative of dynamic interplay between tumour cells and their surroundings when the selective pressure of drug therapy is applied. A more comprehensive understanding of heterogeneity within the tumour cell extrinsic compartments and how this influences resistance development will expand our understanding of treatment responses. Furthermore, the elucidation of stromal-mediated mechanisms of resistance will probably lead to the discovery of new therapeutic options. The targeting of multiple tumour compartments may represent a solution to avoid resistance and achieve durable patient responses; however, elucidating the relationship between tumour heterogeneity and therapeutic response is still a significant challenge.

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Acknowledgements

We would like to express our gratitude to P. Bishop, T. Junttila, K. Leong, J. Settleman, W. Ye, J. Low and S. Scales for their critical review of the manuscript. We also thank the reviewers for valuable insight and suggestions. Our sincere apologies go to authors whose work we are unable to cite due to space limitations.

Author information

Affiliations

Department of Molecular Biology, Genentech, 1 DNA Way, South San Francisco, California 94080, USA.

Melissa R. Junttila & Frederic J. de Sauvage

Competing financial interests

The authors are employees of Genentech Inc. and own shares in Roche.

Corresponding author

Correspondence to: Frederic J. de Sauvage

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Nature ISSN 0028-0836 EISSN 1476-4687