Molecular imaging of cancer microenvironment

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Abstract

In the last decades, researchers have been focusing on cancer cells looking for novel targets, however, tumours grow in a host environment that either contribute to or inhibit tumour expansion and metastatization. Several efforts have been focused on cancer microenvironment for diagnostic and therapeutic purposes. Nuclear medicine can contribute to understand the complexity and role of tumour microenvironment by imaging several of its components (chemokine receptors, immune cells, stromal antigens, vascular factors, etc). In a tumour, each microenvironment component offers many potential targets for several drugs or radiopharmaceuticals. Cancer may be studied using different strategies from different viewpoints: imaging tumour markers or differentiating markers for diagnostic purposes in order to plan personalized therapies (receptor agonists or superagonists); imaging tumour stroma and vascularization to monitor cell adhesion, metastases, angiogenesis and hypoxia; imaging the host response of cancer cells to monitor the efficacy of immunotherapeutic strategies.

Key words: lymphocytes, images, molecules, lymphokines, metastases, diagnosis, therapy, endothelium, growth

Imagen molecular del microentorno del cáncer

Resumen

En las últimas décadas los investigadores han centrado su atención en la observación de las células cancerosas, en búsqueda de nuevos sitios blanco. Sin embargo, el crecimiento del tumor se produce en un entorno que, o inhibe, o contribuye a la expansión del tumor y su metástasis. Varios esfuerzos han estado enfocados al estudio del microentorno del cáncer, con propósitos diagnósticos o terapéuticos. La Medicina Nuclear puede contribuir a la comprensión de la complejidad y del papel que juega el microentorno del tumor, mediante la obtención de las imágenes de varios de sus componentes (receptores de quimioquinas, células inmunes, antígenos del estroma, factores vasculares, etc.). En un tumor, cada componente del microentorno ofrece muchos blancos potenciales para varias drogas o radiofármacos. El cáncer puede ser estudiado mediante diferentes estrategias y enfoques: mediante la imagen de marcadores tumorales, o la diferenciación de estos, con propósitos diagnósticos a fin de planificar terapias personalizadas (receptores agonistas o superagonistas); mediante la imagen del estroma del tumor y la vascularización, para monitorear la adhesión celular, la metástasis, la angiogénesis y la hipoxia; mediante la imagen de la respuesta del huésped de las células cancerosas, con el objetivo de monitorear la eficacia de las estrategias inmunoterapéuticas.

Palabras clave: infocitos, imágenes, moléculas, linfocinas, metástasis, diagnóstico, terapia, endotelio, crecimiento

Introduction

The concept of "microenvironment" has recently gained an important role and it has become the object of several speculations in the last decades in the optic of programming personalized therapies. Tumour growth requires a complex bidirectional interaction between host and cancer cells. As a "parasitic interaction" the cancer cannot exist without the host that provide substances, cytokines, hormones and growth factors that allow malignant cells to take root and spread [1]. The immune surveillance that maintains tissue integrity has a pivotal role in cancer development. Given these premises, the term "immuno-oncology" has been coined and research has been focusing on the concept of cancer microenvironment to clearly understand the underlying mechanisms and to discover novel targets for tailored therapies. In this scenario, nuclear medicine can contribute to clarify the complexity and role of the tumour microenvironment by imaging its components (chemokine receptors, immune cells, stromal antigens, vascular factors, etc). As demonstrated by

the presence of inflammatory cells in biopsies of many cancers, chronic inflammation is known to have a great importance in oncogenesis providing several triggers that increase the risk of cancers [2, 3]. The hallmarks of cancer related inflammation include, of course, cellular and non-cellular elements that compose its microenvironment. This distinction is merely a way to simplify the complex interactions existing between cells and soluble factors produced by cells that exert a paracrine effect on themselves or influence other distant cells. In non-cellular groups we may include growth or inhibiting factors, cytokines that establish a hormonal and biochemical connection between host and cancer. Among the cellular components we should mention fibroblasts, immune cells, stromal cells and endothelial cells that play a pivotal role in promoting neo-angiogenes, thus leading to cancer progression and metastatization [5-8].

During metastatization, different malignant cells are selected in order to escape from tumour surveillance mechanisms, to survive in blood stream and to take roots at distant sites compromising the prognosis of the patient. This process is also the result of complex interaction between malignant clones and host response [1, 2]. One of the main factors involved in lympho-angiogenesis is vascular endothelial growth factor A (VEGF-A), which is mainly produced by endothelial cells but also by mesenchimal cells and fibroblasts. VEGF acts through the interaction with specific receptors expressed on the surface of endothelial cells (VEGFR1 and VEGFR2) [9, 10] promoting the formation of new vessels and hindering the correct diffusion of antitumoural drugs [11].These premises laid the foundation for the development of new targeted drugs like Bevacizumab, an anti-VEGF antibody that prevent VEGF binding to its receptors, thus blocking the synthesis of new vascular and lymphatic vessels. Other drugs, like anti tyrosine kinase inhibitors (TKIs) are able to interfere with VEGFRs signalling. Neoangiogenesis is also indirectly stimulated by the hypoxia inducible factor 1α (HIF-1α) produced in response to hypoxia. Fibroblasts play an important role among the cellular components of cancer microenvironment, since they are able to synthetize different extracellular matrixes through the stimulation of tumour growth factor-β(TGF-β) promoting tumour and vessel growth. Moreover, they can have both stimulatory and inhibitory effects on T-lymphocytes [12]. Other cells that take part in these complex mechanisms are the dendritic cells that have an apoptotic power in cancer cells and produce chemokines that attract for example immune cells natural killer (NK) involved in immune surveillance with high anti-tumour activity [13]. Tumour associated macrophages are important in cancer microenvironment because of their pro or anti-tumour effect. They migrate into cancer and maturate in M1 (anti-tumour effect through the production of pro-inflammatory cytokines for example TNF-α, IL-12) or M2 phenotype (pro-tumour effect through the production of growth factors like VEGF). They also suppress the inflammatory response reducing the effect of antitumour treatments.

Other cellular and non-cellular components may be present in a tumour microenvironment with different roles and functions that have not been completely elucidated yet. Therefore, the present review will focus only on those components which represent potential targets for new drugs or radiopharmaceuticals. Indeed, cancer may be studied using different strategies: imaging tumour or differentiating markers for diagnostic purposes in order to plan personalized therapies (receptor agonists or superagonists); imaging tumour stroma and vascularization to monitor cell adhesion, metastases, angiogenesis and hypoxia; imaging the host response to cancer cells to monitor the efficacy of immunotherapeutic strategies. In the next paragraphs we will describe these three main strategies only focusing on part of the complex network of cancer microenvironment.

Cancer cells (over-expression of specific cancer receptors/targets)

Ligands of cancer specific receptors (receptor agonists or superagonists) may be radiolabeled for diagnostic purposes and in order to plan personalized therapies. A suitable example is provided by thyroid cancer and, in particular, by poorly differentiated (PDTC) or undifferentiated (UDTC) variants that, even if less frequent, are characterized by high death rate (6-10 %) [14]. Well differentiated thyroid tissue expresses sodium/iodide symporter (NIS) on its surface and it is responsible for the uptake of iodine that it is useful for hormonal synthesis. This symporter is also responsible for radio-iodine uptake in the diagnostic and therapeutic fields. Indeed, after surgery the main therapy of differentiated thyroid cancer (DTC) is represented by radio-iodine treatment with ^{131}I that allows the ablation of thyroid remnant or the treatment of distant or loco-regional metastasis. During the process of de-differentiation, tumoural cells lose the expression of NIS and, as a result, they will not uptake iodine anymore. This condition is particularly crucial for both a diagnostic and therapeutic point of view becoming refractory to radio-iodine therapy. ^{18}F-Fluoro-Deoxy-Glucose (^{18}F-FDG) PET/CT still represents the radiopharmaceutical of choice in the follow-up of patients with high serum thyroglobulin (Tg) and negative ^{131}I-whole body scan. Its sensitivity for the detection of distant metastases or local recurrences ranges from 63 % to 98 %, whereas the specificity from 81 % to 100 % [15-18]. However, in the last years several second generation PET radiopharmaceuticals have been developed as alternatives to ^{18}F-FDG, aiming to improve the diagnosis and therapeutic chances of poorly or undifferentiated thyroid cancers [19]. For this purpose [124]I, ^{18}F-FLT, ^{68}Ga-somatostatin analogues, ^{11}C-MET and other PET tracers have been proposed showing promising results that have to be confirmed in wider cohorts of patients and with longer follow-ups. Despite these improvements, no specific diagnostic tools and therapies are available yet for patients affected by undifferentiated histotypes. Therefore, it would be desirable to exploit the relationship between cancer and host microenvironment that may offer a wide set of targets

for molecular imaging and new therapeutic approaches. It could also be crucial to develop new radiopharmaceuticals for early diagnosis of PDTC and UDTC and for therapy decision making. In thyroid cancer, NIS expression is gradually lost, but TSHR is usually retained, even if not functional [20]. Its natural ligand is the TSH, but the endogenous hormone has a relatively low affinity for its receptor. Through scanning mutagenesis, it was possible to synthesise superagonist rhTSH analogues with a 50-fold higher affinity for the TSHR. Such analogues can be radiolabelled to develop promising radiopharmaceuticals to image radio-iodine refractory thyroid cancer metastases. The rhTSH (Thyrogen®) has been radiolabelled with [123]I [21, 22] or [99m]Tc [23] and have been tested in vitro and in vivo with good results. In particular, the superagonist rhTSH analogue, radiolabelled with [99m]Tcby Galli et al. (^{99m}Tc-HYNIC-TR1401), seems to be a promising tool in both pre-operative staging of PDTC and follow-up, but more studies are needed to confirm preclinical results [23].

Tumour stroma: endothelial cells, VEGF, VEGFR

Endothelial cells can be indirectly imaged using radiolabelled VEGF, that as previously described, is an important pro-angiogenic factor (Figure 1).
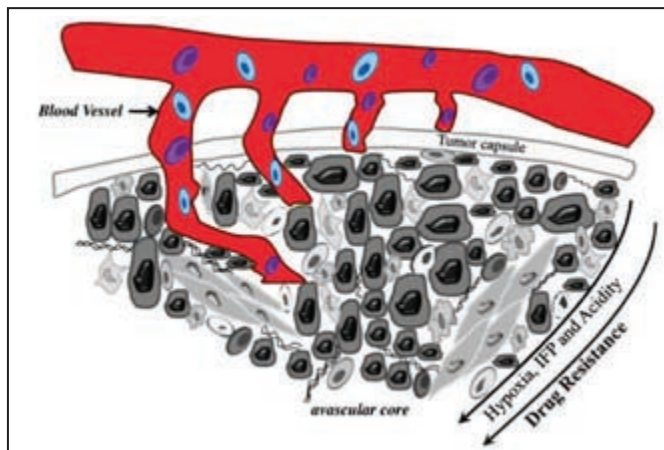

Figure 1. Schematic representation of a cancer lesion with highlighted the blood vessels as potential target for nuclear medicine.

It is classified as a non cellular component of tumoural microenvironment that promotes the growth of endothelial cells deriving from vessels and lymphatics, enhancing vascular permeability and leading to tumour progression and metastatization. These effects are the result of specific pathways activated by the binding of VEGF to its receptors VEGFR1-2-3 and Neuropilin1-2) [9, 10] expressed on both cancer cells and endothelial cells. In the last decades several efforts have been performed in order to develop antiangiogenic therapies that could prevent the binding of VEGF to its receptors (Bevacizumab) or can inhibit tyrosine kinase mediated signalling (Sorafenib). These improvements, together with the increasing attention to personalized therapeutic approach, have led to the development of specific radiopharmaceuticals for the diagnosis and selection of patients eligible to undergo anti-angiogenic treatments in order to predict the response to therapy. Radiolabe-

lled VEGF analogues could be promising radiopharmaceuticals to detect distant metastases of different types of cancers. The most studied variants overexpressed in cancer microenvironment are $VEGF_{165}$, $VEGF_{206}$ and $VEGF_{189}$ mainly located in the extracellular matrix [24]. Another important isoform is $VEGF_{121}$ that has been bradiolabeled with ^{99m}Tc, ^{111}In or ^{123}I, or with positron-emitters to visualize tumour angiogenesis and to monitor therapeutic effects on it [25-31]. Scintigraphic images of ^{99m}Tc-HYNIC-VEGF in rat models, however, showed high uptake of radiopharmaceutical by several organs (mainly kidneys and liver) resulting in low target/background ratio [32]. A more specific uptake by tumour was observed using ^{64}Cu or [68]Ga [33, 34] despite a great uptake from the kidney because of the presence of high concentration of VEFGR in this organ.

Bevacizumab is a recombinant monoclonal antibody that binds to VEGF-A preventing, in turn, its interactions with VEGFRs. This results in an inhibitory effect on tyrosine kinase mediated pathways, blocking the angiogenesis. Bevacizumab received the approval of FDA for the treatment of metastatic tumours, NSLC and glioblastoma. This monoclonal antibody has been radiolabelled, with good results, with both SPECT and PET radionuclides mainly for colorectal and ovarian cancers [35-39].

Host response to cancer: NK cells, B cells, T cells, NK cells

Natural killer cells (NK) are important effectors of immune-surveillance with a marked antitumour activity. Their recruiting in the tumour and their possible use in tumour immunotherapy has been intensively studied (Figure 2). They are a particular subtype of lymphocytes with a CD3^{-}CD56^{+} phenotype and they can be divided in two different subsets with different functions. The CD56^{dim}CD16^{+} phenotype shows marked cytotoxic functions and is predominantly present in the peripheral blood and spleen. The CD56^{bright}CD16^{-} subset, present in lymph nodes, has a regulatory function, producing cytokines in response to IL-12, IL-15 or IL-18 stimulation [13].
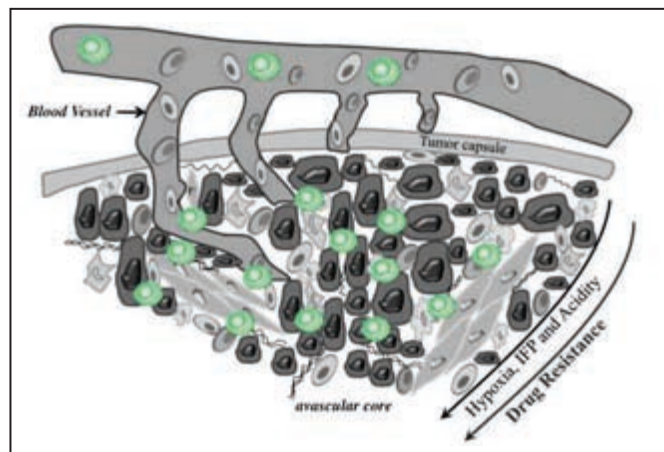

Figure 2. Schematic representation of a cancer lesion with highlighted the immune cells (can be NK or Treg or B-cells or Macrophages) infiltrating the tumour as potential target for nuclear medicine.

NK cell activity is regulated by a balance between inhibitory and activating signals. Under certain stimuli, these cells are able to kill target cells without the need of immunization or MHC restriction. CD56^{dim}NK cells are the most potent cytotoxic subtype able to directly kill cancer cells, but can also produce regulatory factors that enhance the host immune response and indirectly limit tumour growth. Cancer cells may present a reduced or altered MHC-I expression that normally allows them to escape T-cell response. These cells can be directly recognized by NKs through a "missing self" mechanism [40]. Similarly, when cancer cells over express certain ligands secondary to DNA damage or stress, they become targets for NK-activating receptors. NK cells directly kill target cells by intracellular cytotoxic granules containing perforin and granzymes that induce either caspase-dependent or caspase-independent apoptosis. The presence of CD16 on the majority of NK surface is also responsible of a direct antitumour effect through antibody-dependent cellular cytotoxicity (ADCC). Other effects of NK on tumours also involve many cytokines, such as IL-12, IL-2, IL-18 or IFN may also enhance the anti-cancer activity of NK cell.

Their indirect antitumour functions are mediated by cytokines (IFN-γ, TNF-α and IL-10), chemokines and growth factors that target dendritic cells (DCs), T cells, macrophages and endothelial cells [41]. For example, they can drive T cells polarization toward CD8$^+$ cytotoxic phenotype and CD4$^+$ toward T$_H$1 to promote CTL differentiation and are also able to target B cells inducing antitumour antibodies production. Nowadays, many emerging therapies are aimed at increasing the amount of tumour infiltrating NK cells (TINKs). Therefore, imaging of TINKs could allow to image metastases or to follow *in vivo* the efficacy of newly developed drugs [42]. NK based immunotherapies are undergoing pre-clinical and clinical trials. Tumour xenografts from an anaplastic thyroid cancer cell line (ARO), engineered to express IL-12, seem to show a lower proliferation rate than controls ARO cell. In addition, animals seem to have a longer survival enforcing the idea that imaging of TINKs could be useful to evaluate immunotherapy response and for therapy decision making [43]. PET radioisotopes (^{11}C and ^{18}F) have been used for imaging NK trafficking in pre-clinical studies. In fibrosarcoma models [44] NK cells have been radiolabelled with ^{11}C-methyl iodide to demonstrate that positron emission tomography could be useful to quantify the number of effect or cells, which accumulate into tumours and to determine their biodistribution. Another group injected ^{18}F-FDG radiolabeled NKs in HER2/neu positive xenograft models monitoring their trafficking with autoradiography [45]. For Scintigraphic imaging ^{111}In-oxine labelled NK has been studied in metastatic renal carcinoma [46]. Uptake of radiolabelled NK cells, demonstrated by SPECT and also by ^{18}F-FDG-PET, has been reported in 50% of metastatic lesions however high percentage of circulating ^{111}In was released by cells. Furthermore, ^{111}In toxicity negatively influenced NK trafficking into the tumour. Similar findings were obtained in melanoma and colorectal cancer [47,48]. The limitations of *ex-vivo* NK cells radiolabeling can be overcome using anti-CD56 mAb that bind to the immunoglobulin-like adhesion molecule (CD56) expressed on NK surface. The anti-CD56 monoclonal antibody (mAb) was radiolabeled with ^{99m}Tc and administered in animal models with tumour xenograph of thyroid origin previously injected with human NK [49]. Scintigraphic images performed after 24 hours showed that this radiopharmaceutical could image each tumour with higher T/B ratio than the experiments performed with radiolabelled NK. T/B ratio was also correlated with TINKs infiltration through histological evaluation. In conclusion, the radiolabelled anti-CD56 mAb seems to be a promising tool for non-invasive imaging of NK cell trafficking and follow-up of patients undergoing immunotherapies.

Tumour-infiltrating B cells (TIL-B)

Tumour-infiltrating B cells (TIL-B) have been studied most extensively in breast and high grade of serious ovarian cancer, where they are present in about 25 % of tumours and comprise up to 40 % of the tumour infiltrating lymphocyte population [50-53]. In tumour microenvironment they are present together with CD4$^+$ and CD8$^+$ T cells and dendritic cells (DCs) [21-23]. One of the most extensively studied targets to image TIL-B is by means CD20 that it is expressed on their surface and that represents a specific marker of B cells. CD20 is recognised by the mAb Rituximab that is widely used for treatment of non Hodgkin lymphoma (NHL). ^{99m}Tc radiolabelled rituximab is useful for Scintigraphic imaging of NHL. Planar and SPECT images are able to visualize areas of pathologic uptake of radiopharmaceutical identifying recurrences of the disease [54]. Since CD20$^+$ B cells are involved in several autoimmune diseases; ^{99m}Tc-rituximab can be also applied for imaging of patient with rheumatoid arthritis, sarcoidosis and Behcet's disease [55]. From a therapeutic point of view, CD20 is also the target of ibritumomab-tiuxetan (Zevalin®), a ^{90}Y-radiolabelled mAb directed against the same epitope of rituximab, is currently used for treatment of NHL increasing the therapeutic effect of "cold" MoAb [56, 57].

T regulatory cells (Treg)

Naive and activated T cells are able to infiltrate tumours and are subsequently activated through APCs interactions. CD3 is a co-receptor expressed on T cells associated with the TCR. Amongst T lymphocytes, T regulatory cell (Treg), a CD4$^+$CD25$^+$FoxP3$^+$ T cell subtype, play a pivotal role being recruited in tumours by CCL1 and CCL22 ligands. Therefore, targeting such ligands with specific drug may allow stopping Treg recruitment in tumours and enhancing the host immune response. Treg cells infiltrate tumours producing RANKL (inducing metastases) and suppressing the tumour antigen-specific CTLs [58]. They express CD25, a part of IL-2 receptor that is also expressed by NK cells with lower affinity. IL2 can be used as a surrogate marker for imaging activated T lymphocytes (mainly CD8$^+$, CD4$^+$). ^{99m}Tc-IL-2 has been extensively studied for several tumours, in parti-

cular in melanoma [59], hypernefroma [60], squamous cell carcinomas of head and neck [61] showing optimal biodistribution and dosimetry, high T/B ratio and specific targeting to CD25+ cells. Recent reports show that the number of CD4+CD25+FOXP3+ T cells correlates inversely with clinical outcomes in several epithelial carcinomas, including ovarian cancer, breast cancer, and hepatocellular carcinoma. In particular, in melanoma 99mTc-IL-2 seems to provide important prognostic information for selection of patients who may benefit from immunotherapy. Therapeutic efficacy of Ipilimumab has already been demonstrated in many studies, however today a surrogate marker of praecox evaluation of response to therapy is still lacking. This aspect could be fundamental in order to select patients that can continue treatment with Ipilimumab and must undergo others kinds of therapies. 99mTc-IL-2 was used to study 31 patients with cutaneous lesions suspected for melanoma and correlated with histological findings. In 15 of 21 (71 %) melanomas and two of nine (22 %) benign cutaneous lesions, they found uptake of 99mTc-IL-2. The calculated T/B ratios correlated significantly with the number of IL-2R-positive TILs [59]. These results suggest a possible role of 99mTc-IL-2 Scintigraphic in the evaluation of response to immunotherapy. This radiopharmaceutical has also been studied in non-oncologic diseases in particular in the field of autoimmune-inflammatory diseases for example in diabetes mellitus [62, 63], IBD [64, 65], autoimmune thyroiditis [66].

Conclusion

Tumour microenvironment is the result of very complex interactions between host immune system and cancer cells. Many factors of this network can be potentially targeted by several radiopharmaceuticals in order to image molecular mechanisms that underlie tumour progression, to select patients eligible to peculiar therapies, to valuate the response to treatments. Many efforts have been made in the last years in the field of immune-oncology and many others will be performed in next future to improve the knowledge on these aspects aiming at achieving a tailored therapy.

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