Cancer Microenvironment and Cancer Vaccine

Zhen-Yu Ding · Xue-Lin Zou · Yu-Quan Wei

Received: 27 February 2012 / Accepted: 3 April 2012 / Published online: 6 May 2012
© Springer Science+Business Media B.V. 2012

Abstract The cancer microenvironment is constituted of non-transformed host stromal cells such as endothelial cells, fibroblasts, various immune cells, and a complex extra-cellular matrix secreted by both the normal and neoplastic cells embedded in it. The importance of the microenvironment and its potential in cancer therapy is just being established. Among modalities that target the microenvironment, cancer vaccine is a unique strategy which is aimed to elicit specific immunity against components in the microenvironment. Most, if not all, components can be targeted by the vaccines. The most extensively studied are the endothelial cells, fibroblasts and macrophages as well as ECM. Vaccines are in development for each of them. All the vaccines were proved to be effective at providing protective or therapeutic anti-tumor effects in the pre-clinical models. A few of them have been tested in the clinical trials. The mechanisms of the vaccines were mainly related to the cellular immune response such as CD8⁺ cytotoxic T cells, and in some instances CD4⁺ Th cells were involved as well. The present review also discussed the hurdles associated with the microenvironment-based vaccines such as the selection of suitable patients with appropriate biomarkers. With the rapid increase of our knowledge in the cancer microenvironment, the proof-of-concept of microenvironment-based cancer vaccines will surely expand our armamentarium against cancer.

Keywords Endothelial cell, angiogenesis · Fibroblast · Macrophage · Extra-cellular matrix · Vaccine

Introduction

The cancer is composed of not only cancer cells, but other components as well. Maybe it is suitable to consider cancer as discrete “islands” of cancer cells spotted in the “ocean” of supporting structure. The non-cancerous scaffold, or the cancer microenvironment, is constituted of non-transformed host stromal cells such as endothelial cells, fibroblasts, various immune cells, and a complex extra-cellular matrix (ECM) secreted by both the normal and neoplastic cells embedded in it (Fig. 1). The cancer microenvironment is no longer a “bystander”. The significant role played by the cancer microenvironment in cancer biology and cancer therapy is just beginning to be understood.

Scientists pursuit to translate the success of vaccine in the field of contagious disease to that of cancer for a long time. However, serious doubts existed for many years as to whether the immune system is capable of eliminating human cancer. The effort was mostly in vain until the elucidation of the mechanisms behind the immune recognition of tumor cells at the molecular level. T cells, through their T-cell receptors, specifically recognize tumor antigens which are processed and presented as small peptides in the groove of surface human leukocyte antigen molecules [1, 2]. Later it was the milestone discovery that the first tumor specific antigen MAGE-1 was discovered in the 1990s.

Tumor cells produce tumor antigens by secretion, shedding, or tumor lysis, which are then further captured by professional antigen-processing cells such as dendritic cells or macrophages in some instance. These tumor antigens are next processed, presented as small peptides in the groove of MHC molecules for priming and activation of specific lymphocytes. Ultimately, activated antigen-specific CD8⁺ CTLs recognize and lyse corresponding tumor cells (Fig. 2). Lines of evidence indicate that cellular other than humoral immune responses

Z.-Y. Ding · X.-L. Zou · Y.-Q. Wei (✉)
Cancer Center, West China Hospital, West China Medical School,
State Key Laboratory of Biotherapy, Sichuan University,
Guoxue Lane 37,
Chengdu 610041, China
e-mail: yuquawei@vip.sina.com

Fig. 1 A presentation of cancer cells and cancer microenvironment. Cluster of colon cancer cells are in close contact with the surrounding stroma. The ample amount of stroma and complicated components such as ECM and inflammatory cells implicate multiple activities of the stroma in the development of cancer cells

are mainly responsible for tumor rejection [3]. It has been documented that the lymphocytes in host tumor microenvironments are found to be directly cytotoxic to the tumor cells by in situ observations of lymphocyte-tumor cell interaction [4]. The common goal of cancer vaccination strategies is to induce tumor regression through the in vivo activation of tumor-specific T lymphocytes [5]. Usually, the activated T lymphocytes are induced specifically to recognize cancer cells (helper T

Fig. 2 The process and presentation of tumor antigens. Tumor antigens are produced and released from cancer cells, then are up-tok and presented by dendritic cells. Antigen-specific Th1 and Th2 cells are activated by the dendritic cells, whereby cellular and humoral immunity are activated

cells) or to kill them (cytotoxic T cells). With a clearer view of the mutual dependence of cancer cells and its microenvironment, we have now started to gain a deeper insight of the cancer microenvironment that will drive the attention of cancer vaccine to this field. In the review, the focus will be on selected topics with respect to the cancer vaccines related to the cancer microenvironment where substantial progress has been made.

Components of Cancer Microenvironment

The original “seed and soil” hypothesis proposed the microenvironment of a developing cancer was crucial to the carcinogenesis. Recently, we are aware that the process of carcinogenesis is related to the interaction of not only cancer cells and endothelial cells but also the other stromal cells, inflammatory cells and associated extracellular matrix (ECM) [6, 7] (Fig. 3).

Lines of evidence indicate that the growth and persistence of solid tumors and their metastases are angiogenesis dependent [8]. There are also extensive data showing that tumor produces substances to induce neovessel formation from preexisting neighboring blood vessels. One of the hallmarks of cancer is the ability to induce and sustain angiogenesis in the microenvironment [9, 10]. Endothelial cell is at the center of the process of angiogenesis. New vessels formed by endothelial cells supply nutrients and oxygen to support the growth of tumors, but also take away the wastes from the tumor. It is therefore angiogenesis has been the focus of tumor therapy for a long time, and the concept of antiangiogenesis has been widely accepted and entered the clinical practice.

Cancer associated fibroblast (CAF) is a specialized group of fibroblast inside the cancer microenvironment [11]. It serves as a multi-functional component in the microenvironment, actively participating in the regulation of different biological behaviour of cancer such as carcinogenesis [12, 13], metastasis [14, 15], and therapy resistance [16, 17]. CAF constitutes a heterogeneous population of cells from different origins such as normal fibroblast [18, 19], endothelial cells [20], mesenchymal cell [21], or epithelial cells [22].

Tumor associated macrophage (TAM) is gaining focus in the cancer microenvironment [23]. Growing evidence suggests TAM promote tumor growth and progression, instead of fighting against tumor as considered previously [24]. In certain instance such as hypoxia, the TAM polarizes to a type 2 macrophage (M2)-like type [25]. M2-like TAM exerts a profound immunosuppressive effect by secreting and releasing cytokine such as CCL17, CCL22 or CCL24. And regulatory T cells are recruited to the local milieu by these cytokines. Additionally, M2-like TAM promotes angiogenesis through the production of VEGF or EGF [26].

Fig. 3 A schematic presentation of cellular components, ECM and some of the cytokines produced in the cancer microenvironment. The complex interaction among cancer microenvironment and cancer cells through the cytokine network provides potential targets for the cancer vaccines

The endothelial cells, CAF, and TAM are among the most intensively studied cell components in the microenvironment. But there are other cell types that are now getting attention.

The neutrophils are important in the cancer microenvironment. They play paradoxical roles of both promoting cancer destruction and inducing the growth of cancer cells [27]. Cancer cells are known to produce chemotactic cytokines such as the CXCL chemokine subfamily members CXCL8 or CXCL5 that act on the CXCR 1 and/or CXCR2 receptors on neutrophils to recruit the latter [28, 29]. And the neutrophils were found to express and release VEGF, MMP and other cytokines including CXCL8 and CXCL1, all of which contribute to the invasion of endothelial cells and vessel formation. The angiogenesis follows and cancer progression is enhanced.

Another cell needs to focus is the adipocyte. A prospective survey of the American population of more than 900,000 established the obesity was associated with increased death rates for all cancers combined [30]. The International Agency for Research on Cancer has classified the causal link between obesity and cancers of the colon, female breast (postmenopausal), endometrium, kidney (renal cell), and esophagus (adenocarcinoma). And it suggests that overeating may be the largest avoidable cause of cancer in nonsmokers [31]. However the contribution of adipocytes to carcinogenesis is just begun to be unveiled. Adipocytes produce a panel of biological active substances related to the angiogenesis and tissue inflammatory [32]. For instance, the proinflammatory factors $\text{TNF-}\alpha$ [33] and IL-6 [34] produced by adipocytes activate the $\text{NF-}\kappa\text{B}$ [35] and STAT3[36]inflammatory pathways respectively. Besides, adipocytes release a panel of proinflammatory hormones such as leptin or adiponectin and these hormones are thought to play a role mediating oncogene pathway activation [37].

The ECM which is a key component of the cancer microenvironment is now widely accepted as a critical regulator of

cancer progression [38]. The ECM is in intimate contact with cancer cells, and as a source provides lots of cytokines to support the growth, survival, motility, and angiogenesis of cancer cells. Apart from cytokine production, the contact of ECM with cancer cells activates the intra-cellular signaling pathways which further lead to cell cycle progression, migration, and differentiation.

Vaccines Targeting Angiogenesis: Endothelial Cell Vaccine and Beyond

Numerous antiangiogenesis drugs have been tested for the anti-tumor effects, and some are in the clinical use now [39]. Besides angiogenesis inhibitors, anti-angiogenic vaccines with the elicitation of specific antibodies and cells are promising strategies (Fig. 4). We proposed that the breaking of immune tolerance against autologous angiogenic, endothelial cells should be a useful approach for cancer therapy. Our study

Fig. 4 A presentation of the concept of the “endothelial vaccine”. Vessels support the growth of amounts of cancer cells. The cancer vaccines targeting vasculature by eliciting antibodies and cytotoxic T cells against endothelial cells instead of cancer cells

showed that immunotherapy of tumors using fixed whole endothelial cells as a vaccine was effective in affording protection from tumor growth, inducing regression of established tumors and prolonging survival of tumor-bearing mice. Furthermore, autoreactive immunity targeting to microvessels in solid tumors was induced and was probably responsible for the anti-tumor activity. In an attempt to explore the possible mechanism by which anti-tumor activity was induced with the endothelial cells, we found that humoral immunity might play a pivotal role in our system. Immune mechanism analysis revealed the requirement for CD4⁺ T lymphocyte in the induction of autoimmune response against autologous angiogenic cells [40].

After our publication, the concept of “endothelial cell vaccine” inspired interest in other groups as well. Another group used the human viable umbilical vascular endothelial cell as a vaccine [41]. In their study, both humoral and cellular immunity were elicited against endothelial cells. Notably, the adoptive transfer of T cells eliminated tumors in 4 from 6 tumor-bearing mice. Considering the notorious reputation of Lewis lung cancer model for its unresponsiveness to treatment, the results were amazing and promising. Okaji Y et al. from Japan used the syngeneic endothelial cell-based vaccine (hepatic sinusoidal endothelium isolated from BALB/c mice) and they found the vaccine arrested pulmonary metastases in a murine colon cancer model in both protective and therapeutic settings [42]. Inhibitory antibodies involving IgM and IgG subclasses were detected and these antibodies had a strong affinity for antigens of both murine and human endothelium. Flow-cytometry and chromium-release cytotoxicity assay revealed a specific cytotoxic T cell response against endothelial cells, which were lyzed in an effector: target ratio-dependent manner. Neither antibodies nor cytotoxic T cells reacted with cancer cells.

Scappaticci FA et al. from America also confirmed that endothelial cells can be used as a vaccine in preclinical models [43]. In their report, three endothelial cell vaccine preparations from syngeneic, allogeneic and xenogeneic sources were used to vaccinate mice. All mice developed humoral immune responses to endothelial cells and showed lower basal serum VEGF levels compared with unvaccinated control mice. Mice receiving the syngeneic vaccine showed substantial inhibition of tumor growth after B16F10 melanoma challenge. The developed tumors had lower microvessel density counts compared with the other groups.

Recently another report from Japan tested the efficacy of a vaccine prepared from a transformed endothelial cell line [44]. Vaccination inhibited subcutaneous melanoma growth and appearance of lung metastasis compared to control. Survival period was elongated in the vaccination groups in both of the two models. Specific antibodies to the syngeneic endothelial cell vaccine were elicited.

A pilot clinical trial has tested the efficacy of the endothelial cell vaccine in the clinical setting [45]. The vaccine was prepared from glutaraldehyde-fixed human umbilical vein endothelial cells and administered every week for the first month and then every 2 weeks. Totally nine patients with cancer were recruited. Both specific antibody and cellular reaction were detected. Magnetic resonance imaging showed objective responses in three patients with malignant brain tumors, while the three patients with colorectal cancer showed no response. No serious adverse effects were observed, except for a DTH-like skin reaction at the injection site.

Compared to whole cell vaccine, the specific vaccine targeted to a more specific antigen may provide stronger immune reaction, less off-target effects, and also with more clear mechanisms underlying. During the process of angiogenesis, many cytokines play critical roles in the “node” steps and are considered “driver factors”. And lots of these factors have been proposed as valuable antigens for the development of cancer vaccine. It should be of notice that this kind of cancer vaccine is a little bit deviation of traditional cancer vaccines which recognize antigens on the surface of cancer cells. This novel kind of cancer vaccine targets antigens presented on the stromal cells instead. A panel of angiogenesis related factors has been tested in this way and many have been identified as potential targets.

VEGF-VEGFR Axis

The most potent pro-angiogenic factor discovered thus far is the vascular endothelial growth factor (VEGF). It recognizes and binds the corresponding receptor (VEGFR) on the surface of the endothelial cells and promotes the growth of the latter. The VEGF-VEGFR axis is critical for the angiogenesis [46]. Therefore, the studies on vaccines targeting this axis are out-numbered than the others.

The earliest report on cancer vaccine on VEGFR may be dated back to the year of 2002. Li Y et al. adopted a dendritic cell (DC) vaccine which was pulsed with VEGFR2 protein from mouse [47]. This DC vaccine efficiently inhibited the growth of lung metastasis in murine melanoma and lung cancer models. Alginate assay proved angiogenesis was significantly inhibited with the vaccine. The *in vivo* depletion experiments demonstrated the CD8⁺ cytotoxic T cells were mainly responsible for the anti-tumor and anti-angiogenesis effects.

Soon after the first publication, another report got publication in the journal of Nature Medicine [48]. This group used a bacterial (attenuated *S. typhimurium*) vaccine carrying DNA for VEGFR2. This oral vaccine was tested for its anti-tumor efficacy in a panel of tumors including murine

melanoma, colon cancer, and non-small cell lung cancer. The lung metastases and the angiogenesis were inhibited significantly. Besides, the cytotoxic T cells from the immunized mice lysed the endothelial cells expressing VEGFR2. Later on, the authors adapted an alternative strategy whereby a DNA vaccine based on VEGFR minigene instead of the whole gene was constructed [49]. In this study, a specific H-2D restricted epitope recognized by cytotoxic T lymphocytes was screened out from both the minigene and whole-gene vaccine. Such epitope-specific T cells were proved to be capable of specifically killing endothelial cells. As a result, long-lasting anti-angiogenic effects were achieved. In another report, the same authors tested the VEGFR2 minigene vaccine the mouse models of tumors of different origins [50]. The authors argued the minigene vaccine performed as well as the whole gene vaccine through similar mechanisms and provided a more flexible alternative for future development.

We also tested the idea of VEGFR vaccine. In our study, we prepared a VEGFR-2 protein vaccine. We found that immunotherapy with the vaccine was effective at both protective and therapeutic antitumor immunity in several solid and hematopoietic tumor models in mice. The humoral immunity was mainly responsible for the anti-tumor effects. Besides, the anti-sera from the immunized mice inhibited the proliferation of endothelial cells. The depletion of *in vivo* CD4⁺ T cells abrogated the anti-tumor effects of the vaccine [51]. In addition, we used human VEGFR2 vaccine to test its anti-tumor effects [52]. This DNA vaccine elicited a strong, protective and therapeutic anti-tumor immunity through an anti-angiogenesis mechanism in mouse models.

VEGF is another candidate for the design of vaccine to block the VEGF-VEGFR axis. To this end, we prepared a plasmid vaccine against VEGF [53]. This vaccine provided both prophylactic and therapeutic anti-tumor immunity in breast cancer, sarcoma, and hepatoma models in mice. The specific anti-VEGF antibodies were separated from the sera of the vaccinated mice, and inhibited the proliferation of endothelial cells *in vitro*. The adoptive transfer of the sera inhibited the angiogenesis *in vivo*. The CD4⁺ T cells played a central role in mediating the anti-tumor and anti-angiogenesis effects.

FGF2-FGFR Axis

Basic fibroblast growth factor (bFGF, or FGF2) has been widely accepted for its pro-angiogenic effects. The interaction between FGF2 and its receptor FGFR triggers downstream signals which “turn on” the angiogenic switch. We tested the idea of cancer vaccine on this axis as well. Our results showed that vaccination with a FGFR-1-based DNA

vaccine was effective at antitumor immunity in three murine models [54]. Furthermore we detected FGFR-1-specific autoantibodies in sera of immunized mice. And the purified immunoglobulins were mainly responsible for the inhibition of endothelial cell proliferation *in vitro* and the antitumor activity *in vivo*. In addition, the CD4⁺ T cells were important for the production of FGFR-1 antibodies. Is that possible the bFGF, the ligand for FGFR-1 as a potential target for cancer vaccine as well? This is an interesting question that remains elusive.

Other Angiogenesis Related Factors

Cancer vaccines targeting other angiogenesis related factor were tested as well. The cancer vaccine, no matter in the form of protein, DNA, or genetically modified whole cell vaccine, targeting important factors including Tie2 [55], Endoglin [11], EGFR [12] (Fig. 5), PDGF [13], or CXCL10 [14], all achieved anti-angiogenic and anti-tumor effects. Both cellular and humoral immune responses were involved, depending on the specific molecules, and even more critically, the route of administration and component of the vaccine. Ample data have been provided to confirm the cancer vaccine against angiogenesis inside the cancer microenvironment is both feasible and practical.

Fig. 5 Usually growth factors bind and activate EGFR on the cell membrane of cancer cells to stimulate the down-stream PI3K-Akt-mTOR and Ras-Raf-MEK pathway. Gene transcriptions and cell viability are enhanced. However the lymphocytes and antibodies elicited by EGFR-based vaccine block the binding of growth factor and EGFR

Vaccines Targeting Fibroblast in the Microenvironment

As the roles of CAF in cancer pathogenesis and progression being gradually established, the therapy strategy based on CAF has been proposed. For example, a monoclonal antibody was used to block the activity of tenascin-C which is an important factor on the surface of CAF to drive the progression of cancer cells [56]. The anti-tenascin antibody is being evaluated in clinical trials by now [57]. It is thus reasonable to target CAF by the strategy of cancer vaccine as well.

Fibroblast activating protein (FAP) is a serine protease synthesized by the CAF. FAP is exclusively expressed in the CAF [58] and over-expression of FAP promotes tumor progression and metastasis [59]. It is therefore implicated a potential therapeutic target in the cancer microenvironment [60]. A specific antibody against FAP has been developed and now is undergoing clinical evaluation [61].

Alternatively, Loeffler M et al. adopted a vaccine to target the FAP as a tumor associated antigen [62]. In this study, the researchers constructed an oral DNA vaccine targeting FAP. The administration of the FAP vaccine conferred resistance to the growth of murine colon cancer and breast cancer in the prophylactic setting. In the therapeutic setting, the vaccine significantly inhibited the growth of lung metastases. Furthermore, the authors demonstrated that it was the CD8⁺ T cells mediated the anti-tumor immunity. The administration of both the vaccine and chemotherapy agents enhanced the uptake of the chemotherapy drugs by the tumor and led the tumor rejection.

Later, this group made further attempts to explore the mechanisms underlying the anti-tumor immunity by this FAP-based vaccine [63]. In this study, they used a murine orthotropic breast cancer model. When mice with tumor burden received FAP vaccine together with chemotherapy, there were significant anti-tumor and metastasis-inhibiting effects. Then they assayed the cytokine profile in the cancer microenvironment, and found a Th1 polarization, as well as CTL activation. The immune suppressors such as tumor associated macrophages (TAM) and myeloid derived suppressor cells (MDSC) were eliminated while the immune effector cells such as dendritic cells were recruited. The angiogenic and lymphogenic potential were also reduced by the vaccine. The authors concluded the elimination of CAF by cancer vaccine showed promise for cancer therapy.

We used a similar strategy by using a DNA vaccine targeting FAP [64]. The mice were immunized subcutaneously with a complex of DNA vaccine and liposome. The administration of the DNA vaccine suppressed the growth of tumor and prolonged the life span of the mice with tumors. Similar with the previous report, we found CD8⁺ T cells were critical for the anti-tumor immunity. Adoptive transfer of the CD8⁺ T cells significantly inhibited the growth of tumors in recipient mice.

In another study, the group led by Gilboa attempted to screen for candidate antigen from the cancer microenvironment by transfecting in vitro produced dendritic cells with a panel of potential antigens such as MMP9, MMP14, Survivin, and FAP [65]. The results definitely show the CTL response is induced against FAP, but not others. Substantial CD4⁺ and CD8⁺ immunity were elicited with a dendritic vaccine transfected with a fusion molecule consisting FAP and LAMP. The same group conducted another study [60]. In this report, the authors used the same vaccine of dendritic cells transfected with fusion molecules of FAP and LAMP and tested the anti-tumor efficacy in several murine tumor models. The tumor growth was significantly inhibited and the magnitude of anti-tumor immunity was comparable to that of a vaccine targeting tumor associated antigen. This study suggested the FAP could serve as a useful tumor rejection antigen in a broad range of cancers.

Connective tissue growth factor (CTGF) is another important factor produced by CAF. CTGF belongs to the cysteine-rich protein 61/CTGF/nephroblastoma overexpressed gene (CCN) family [66]. The CCN family constitutes a variety of secreted, matrix-associated proteins that are involved in the tumor growth. A growing body of evidence shows that CTGF expression is associated with tumor development and growth [23]. In order to block the CTGF activity, researchers have developed the monoclonal antibody specific for the CTGF and the antibody showed promising results in the preclinical mouse models [24]. In this sense, cancer vaccine would be another strategy to block the CTGF for the cancer therapy. However, till now to our best knowledge, there are no reports on this concept.

Researchers took advantage of the pro-survival attributes of CTGF and constructed a fusion DNA vaccine which was composed of CTGF and a model tumor antigen E7 [25]. The CTGF/E7 vaccine prolonged the survival of dendritic cells in vivo and thus enhanced the CD4⁺ and CD8⁺ T cells response. Impressive anti-tumor effects against E7-expressing tumors were observed and were attributed to the higher number of E7-specific CD8⁺ T cells induced by the fusion vaccine. Later, the same group tried the combinatory immuno-chemotherapy with the CTGF/E7 vaccine and chemotherapy agent paclitaxel [26]. In this study, the authors found the chemotherapy drug paclitaxel given at the metronomic low doses, but not the maximal tolerable doses, were synergistic with the antigen-specific CTGF/E7 DNA vaccine. Paclitaxel enhanced the vaccine's potential to delay tumor growth and decreased metastatic tumors. The possible mechanisms underlying were the depletion of regulatory T cells and inhibition of angiogenesis.

Interestingly, unlike the endothelial cell vaccine, there are no study on the whole CAF cell vaccine in preclinical models, although accumulating data indicate CAF has unique antigens on the cell surface and CAF plays

a critical role in the tumor carcinogenesis and progression. The reasons, we suppose, may be due to the wide spread of fibroblasts in the body and general anergy of immunity (tolerate) to the fibroblasts. While in the case of angiogenesis, there are only occasions such as wound healing or menstruation in women when angiogenesis takes place. This implies the immune tolerance to CAF is more difficult to break.

Vaccines Targeting TAM

As growing data underline the important roles by TAM during carcinogenesis and cancer progression, it is proposed that the TAM emerges as a new potential therapeutic target for cancer. A variety of pre-clinical studies were performed to test the concept of TAM-based anti-cancer therapy and also some studies try to unveil the mechanisms of the clinical available drugs which may be related to TAM to some extent. For example, researchers tested the receptor antagonist of CCL5 for its anti-tumor effects [67]. The antagonist blocked the activity of CCL5 and recruitment of TAM thereafter. The antagonist showed significant anti-tumor effects in a murine breast cancer model. Till now there are no available agents specific for TAM in clinical use. But some drugs have been found to be able to target TAM as well. Biphosphonate such as zoledronic acid has been used for cancer-associated skeletal complications for a long time. But only now we begin to realize the biphosphonates have effects on TAM as well which contributes to its anti-tumor effects [68].

Only recently researchers began to focus TAM as a target for the cancer vaccines. This area is a state-of-art but ever-growing field. In 2006, Luo Y et al. presented an oral DNA vaccine which was believed to target TAM [69]. The DNA vaccine was based on legumain which is over-expressed on TAM. The administration of the DNA vaccine in mouse induced a robust CD8⁺ T cell response against TAM. The distribution of TAM in cancer microenvironment was reduced and the production and release of pro-angiogenic factors such as MMP-9, TGF- β , and VEGF from TAM was inhibited. As a sequence, the growth and metastases of a variety of tumors were significantly inhibited.

Beside the full length legumain, the authors adopted an oral minigene vaccine encoding the Legumain H-2D or 2K epitopes [70]. The vaccine vectors were transformed into attenuated *Salmonella typhimurium* for oral delivery. The vaccine effectively protected the mouse against lethal challenge of tumor cells. In addition, a robust CTL response against legumain-positive TAM was induced and angiogenesis was significantly inhibited. Most importantly, the minigene vaccine was as effective as the full length gene vaccine. The study, although conducted by the same

authors, provided further evidence in support of the proof-of-concept that TAM can be targeted for cancer therapy.

Unfortunately, there were merely limited reports on the cancer vaccine against TAM. We think this might be a reflection of the current status of TAM research. There is an urgent need to understand the lineage development for TAM and identify the critical factors therein that can be targeted by cancer vaccine. Considering the rapid accumulation of knowledge on TAM, we foresee a boost of vaccine based on TAM in the next decade.

Other Vaccines Targeting Cancer Microenvironment

Increasing evidence supports the notion that neutrophils play a role in the tumor progression, and efforts are now undertaking to reduce the number or biological activity of the neutrophils in the cancer microenvironment. Such a concept has been proved in a preclinical murine model where neutrophils in the microenvironment were reduced by a cytotoxic antibody [71]. In this study, the inhibited angiogenesis was observed. It is reasonable to develop neutrophil-targeting vaccines which would achieve the same goal. However, given the importance of neutrophils in the innate immune system, this vaccine would never be fulfilled. Maybe it is suitable to develop vaccines that would block the process of neutrophil recruitment into the cancer microenvironment by targeting chemokines or their receptors.

It is reasonable to speculate that cancer vaccine targeting adipocyte might be feasible and intriguing. But till now there are merely limited reports supporting this idea, possibly due to the reason that we cannot differentiate cancer associated adipocytes and “normal” adipocytes in a feasible way. Heid HW et al. from German developed a novel peptide vaccine based on the antigen adipophilin. It was noteworthy the antigen adipophilin was initially identified as an adipocyte-specific antigen [72]. In this report, the peptide vaccine elicited cytotoxic immunity against a variety of cancer cells expressing endogenous adipophilin such as renal cell carcinoma, breast cancer, melanoma, and multiple myeloma etc. [73]. However it remained obscure whether the induced cytotoxic immunity targeted the adipocyte as well, or whether the immunity contributed the anti-tumor effects if this was the case.

The complex interplay between the cancer cells and the ECM may play a pivotal role in the process of carcinogenesis and cancer progression [74]. The extracellular matrix remodeling proteinase such as the matrix metalloproteinase (MMP) is the principal mediators of the alterations in this process [75]. The notion of MMP as therapeutic target was proposed long ago [76]. We used the strategy of cancer vaccine to inhibit endogenous MMP-2 [77]. We found that

the vaccine based on MMP-2 induced both protective and therapeutic antitumor immunity. Autoantibodies against MMP-2 in sera of immunized mice were substantially increased in response to the immunization. The elevation of MMP-2 in the sera was abrogated with the vaccination. Transmigration of human endothelial cells and tumor cells through gelatin-coated filters was inhibited with immunoglobulins isolated from immunized mice. The gelatinase activity of MMP-2, including both latent MMP-2 (Mr 72,000) and active MMP-2 (Mr 66,000) derived from tumor tissues, was apparently inhibited by the vaccination. Later we changed the strategy to the cell vaccine by transfecting murine colon cancer CT26 cells and lung cancer LLC cells with chicken MMP-2 cDNA constructs. MMP-2-specific autoantibodies in sera and tumor cells were found in mice after immunization. Protection against tumor growth was evaluated in respect of the relative contributions of autoantibodies, CD4⁺, and CD8⁺ T cells. Treatment with this vaccine prolonged the survival time of mice bearing cancer. The specific cytotoxic T-cell responses suggested that the treatment increased CD8⁺ T-cell activity. The antitumor activity was abrogated by in vivo depletion of CD4⁺ and CD8⁺ T-lymphocytes and improved by adoptive transfer of CD4⁺ and CD8⁺ T-lymphocytes [78].

Cells respond to the mechanical and biochemical changes in ECM through the crosstalk between integrins and the actin cytoskeleton [79, 80]. The family of integrins mediates the cell migration, growth, differentiation, and apoptosis, and not surprisingly it has been proposed as a potential target for cancer therapy [81]. We tested a DNA vaccine encoding the ligand-binding domain of chicken integrin $\beta 3$ for its anti-tumor activity in several tumor models in mice. In both protective and therapeutic immunity models, the vaccine demonstrated anti-tumor activity through induction of CD4⁺-dependent antibodies. Purified immunoglobulins against integrin $\beta 3$ integrin inhibited endothelial cell proliferation in vitro and possessed anti-tumor and anti-angiogenic activity by adoptive transfer in vivo [82].

Prospectives

The past century has witnessed rapid progress in the field of cancer treatment. Surgery and radiotherapy have become mainstays for the treatment of locoregional disease, followed by increasingly effective chemotherapy for disseminated disease. However, despite the great effort that has been devoted, cancer continues to exert a great threat to humankind. The prognosis is still discouraging for most cancer patients. In addition, radiotherapy and chemotherapy kill tumour cells in a non-specific way and inevitably cause toxicities while reducing patient tolerance. Therefore, there is a high but unmet need to develop and implement

innovative approaches to cure cancer. Fortunately, in recent years, our treatment armamentarium has expanded beyond conventional treatment modalities to include immunotherapy, which acts against cancer in a more specific way [83].

A great increase of our knowledge about the cancer immunogenicity and the immune response of our body has been achieved, and the leap has been translated into the development of new cancer immune therapies. Great progress has been achieved in the cancer immunotherapy [84]. For example, the monoclonal antibody against the tumor antigen HER-2 which is overexpressed in breast cancer or gastric cancer (Trastuzumab) has been widely used in clinical practice. Additional therapeutic antibodies include Rituximab against CD20 in B-cell lymphoma and Cetuximab against overexpressed EGFR in colon cancer, head and neck cancer or lung cancer. Also numerous clinical trials began to evaluate the efficacy of adoptive T cell transfer for a panel of tumors [85, 86]. Maybe in the future not so far, some of the immune cell therapies will become a part of the bed-side practice.

The potential of cancer vaccine has been gradually established after decades of exploration. The first approved cancer vaccine Melacine for malignant melanoma became available in Canada in the year of 2000 [87]. Then the melanoma vaccine M-Vax in Australia and the renal cancer vaccine Oncophage in Russia came into market. In April 2010 came the milestone vaccine Provenge (Sipuleucel-T) for prostate cancer. Provenge is based on the fusion of PAP which is a tumor marker for prostate cancer and GM-CSF. Provenge provides the patients with advanced prostate cancer a survival benefit of 4.2 months. Over 100 cancer vaccines are in phase II/III clinical trials now and surely some of them will become parts of our routine clinical practice [88] (Table 1).

Most of the cancer vaccines are to target the TAAs on the cancers cells. However due to the genetic instability of cancer cells, the cancer cells always “figure out” ways to evade the immune system such as by down-regulation of MHC expression, sequestration of TAAs, or recruitment of immunosuppressive components. Compared with the cancer cells, the cell components of cancer microenvironment provide an alternative target for the cancer vaccine. The cancer microenvironment is more stable, less likely to develop genetic mutation which brings immune anergy. Besides, it is more easily to get access by immune effector cells. Thus it is promising to develop cancer vaccines targeting cancer microenvironment instead of cancer cells directly.

Because of the short period of development, the cancer microenvironment vaccine is generally at its early stage. Most of the studies were conducted at the pre-clinical stage, except very limited number of clinical trials. It seems a long way to go for the microenvironment vaccine. However, we think this strategy will be translated into clinical practice

Table 1 List of cancer vaccines in the late stage of development (1. J Clin Oncol, 2008; 26: 655; 2. N Engl J Med, 2010; 364: 2119; 3. Clin Cancer Res, 2010; 16:5539; 4. J Clin Oncol, 2005; 23:6854; 5. J Clin Oncol, 2006; 24:3089; 6. N Engl J Med, 2010; 363:411; 7. 2009 Genitourinary Cancers Symposium)

Vaccine	Component	Cancer type	Clinical trial phase	Effects
Vitespen (Oncophage)	autologous, tumor- derived heat shock protein gp96 peptide complexes	melanoma	Phase 3	overall survival is statistically indistinguishable from that in the control arm [1]
–	gp100: 209-217(210M)	melanoma	Phase 3	Overall survival, progression-free survival, response rate were all improved with the vaccine [2]
TroVax	modified vaccinia Ankara encoding the tumor antigen 5T4 (MVA-5T4)	renal cell cancer	Phase 3	No difference in overall survival in the overall study population [3]
–	Bec2/bacille Calmette-Guerin (BCG)	Small cell lung cancer	Phase 3	No improvement in the overall survival, progression-free survival or quality of life with the addition of the vaccine [4]
Provenge (Sipuleucel-T)	antigen-presenting cells pulsed with a fusion protein which consists of PAP and GM-CSF	Prostate cancer	On the market	Improved overall survival and progression free survival [5, 6]
GVAX	GM-CSF transduced allogeneic prostate cancer cells	Prostate cancer	Phase 3	survival was not significantly improved compared to chemotherapy [7]

someday in the future just like the cancer vaccines (Table 1) do these days. We believe this process can be accelerated if the following issues are improved.

The immune capabilities of cancer patients especially those with advanced cancer are often compromised from the consumption by the cancer or poor nutritious status (cachexia). It is optimal for the microenvironment vaccine to be administered on patients at earlier stage. Not surprisingly, the current ongoing trials on lung cancer that target antigen MAGE (MAGRIT) or MUC1 (INSPIRE) are both performed in patients with postoperative or local advanced lung cancer.

The real problem is we still lack an efficient and easy way to measure the specific anti-tumor immunity *in vivo*. Currently the means most widely accepted include the ELISPOT to measure the humoral response or the MHC-peptide tetramer analysis to measure the T cell response. These techniques are difficult, time-consuming and hard to be performed in everyday practice. We need to expand our detection methods, and are to develop new methods to detect these immune changes. For example, now we use the Positron Emission Tomography (PET)-CT to scan the whole body for the foci of abnormal metabolism which usually stand for cancer. Can we adopt new ways such as functional imaging to monitor the real-time anti-tumor immunity? If we can develop a suitable tracer, this concept will soon come into reality.

Another hurdle is to look for suitable surrogate markers or biomarkers that can predict the efficacy of the microenvironment-based cancer vaccine. From our past experience, we know the success of targeted therapies including cancer vaccine always depends on the existence of suitable targets. For example, without the guidance of EGFR gene mutation status, clinical trials with EGFR tyrosine kinase inhibitors (TKIs) most often

ended up with negative results. Now we select the lung cancer patients harboring the EGFR mutation for the administration of TKIs. In this population, the TKIs far out-perform the chemotherapy. But for the most of the stroma-directed therapies, we still don't have appropriate biomarkers to select the suitable patients. The defects in our knowledge will undoubtedly exert impacts on the development of microenvironment directed cancer vaccines. The clinical trials conducted in unselected patients will probably veil the success in the certain subgroup of patients and mislead us to the wrong conclusion.

If we find some effective microenvironment directed cancer vaccines, then how can we combine the vaccines with the currently available therapies to maximize the benefit of each modality alone? What we learned is that the sequential administration of each therapy in the right order and the correct time window are critical to achieve the optimal effects. The data from our lab showed when the chemotherapy and anti-angiogenesis therapy are used together, it is appropriate the chemotherapy should be administered followed by antiangiogenesis therapy or both are used at the same time [89]. We believe the same trial-and-error principle will probably be applicable to other combinations also.

Conclusions

The cancer microenvironment has never been so emphasized before. Now we clearly understand the cancer microenvironment is not only a supporting structure for the cancer cells, but also provides the oxygen and nutrients to the latter, as well as growth factors to promote the cancer progression and protease to degrade the matrix to enhance the process of metastases.

The importance of the cancer microenvironment therefore opens a new “window” for the intervention of cancer therapy.

After decades of efforts, the immunotherapy of cancer has been gradually established as one of the treatment modalities. The adoptive transfer of monoclonal antibodies has achieved definite effects in clinics and we believe the booming of monoclonal antibodies will persist. The adoptive transfer of immune effector cells or T cell therapy to some extent has unique mechanisms of action and achieved amazing effects in some cases. As far as the cancer vaccine is considered, it is in the stage of rapid rise. Several cancer vaccines have been approved, especially for patients with melanoma or prostate cancer. Given the rapid progress in the field of cancer immunotherapy, we think the development of microenvironment-directed cancer vaccine will provide an additional opportunity to the cancer therapy.

The “ocean” of the cancer microenvironment is so complex that we can hardly tell precisely how many different kinds of cell types or factors are there. But roughly, the microenvironment is composed of cell compartments and the matrix. The endothelial cells, CAP, and TAM are among the most important cell types in the cancer microenvironment. And now there are cancer vaccines being developed against each of these cell types. The vaccines against endothelial cells were developed most early and are relative mature. Some of the vaccines have been tested in the pilot clinical trials.

The aim of microenvironment-directed cancer vaccine is to elicit a specific immunity against components in the cancer microenvironment. It should be noted the immune cells are also important players in the cancer microenvironment. Actually, the strategy tries to turn one component against another in the same microenvironment. This is an extremely challenging attempt, we would say. Some components inside the cancer microenvironment such as regulatory T cells or MDSCs although important are hard to be targeted by cancer vaccines. But with the help from the elucidation of interactions among cells in the cancer microenvironment, this brand-new strategy will probably bring us an unprecedented opportunity of attacking not the cancer cells but the cells that supports them.

Acknowledgements The project was partially supported by grants from National Natural Science Fund of China (30901756) and Doctoral Fund of Ministry of Education of China (20090181120100).

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Bjorkman PJ, Saper MA, Samraoui B et al (1987) Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329:506–12
2. Bjorkman PJ, Saper MA, Samraoui B et al (1987) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329:512–8
3. Monsurò V, Nagorsen D, Wang E et al (2002) Functional heterogeneity of vaccine-induced CD8(+) T cells. *J Immunol* 168:5933–42
4. Wei Y, Zhao X, Kariya Y et al (1996) Induction of autologous tumor killing by heat treatment of fresh human tumor cells: involvement of gamma delta T cells and heat shock protein 70. *Cancer Res* 56:1104–10
5. Lizée G, Cantu MA, Hwu P (2007) Less yin, more yang: confronting the barriers to cancer immunotherapy. *Clin Cancer Res* 13:5250–5
6. Bissell MJ, Labarge MA (2005) Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? *Cancer Cell* 7:17–23
7. Bhowmick NA, Neilson EG, Moses HL (2004) Stromal fibroblasts in cancer initiation and progression. *Nature* 432:332–7
8. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 82:4–6
9. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100:57–70
10. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144:646–74
11. Lee SH, Mizutani N, Mizutani M et al (2006) Endoglin (CD105) is a target for an oral DNA vaccine against breast cancer. *Canc Immunol Immunother* 55:1565–74
12. Lu Y, Wei YQ, Tian L et al (2003) Immunogene therapy of tumors with vaccine based on xenogeneic epidermal growth factor receptor. *J Immunol* 170:3162–70
13. Kaplan CD, Krüger JA, Zhou H et al (2006) A novel DNA vaccine encoding PDGFRbeta suppresses growth and dissemination of murine colon, lung and breast carcinoma. *Vaccine* 24:6994–7002
14. Li G, Tian L, Hou JM et al (2005) Improved therapeutic effectiveness by combining recombinant CXC chemokine ligand 10 with Cisplatin in solid tumors. *Clin Cancer Res* 11:4217–24
15. Liotta LA, Kohn EC (2011) The microenvironment of the tumour-host interface. *Nature* 411:375–9
16. Olumi AF, Grossfeld GD, Hayward SW et al (1999) Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* 59:5002–11
17. Hayward SW, Wang Y, Cao M et al (2001) Malignant transformation in a nontumorigenic human prostatic epithelial cell line. *Cancer Res* 61:8135–42
18. Fidler IJ (2003) The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. *Nat Rev Cancer* 3:453–8
19. Karnoub AE, Dash AB, Vo AP et al (2007) Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449:557–63
20. Crawford Y, Kasman I, Yu L et al (2009) PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. *Cancer Cell* 15:21–34
21. Blish CA, Nguyen MA, Overbaugh J (2008) Enhancing exposure of HIV-1 neutralization epitopes through mutations in gp41. *PLoS Med* 5:e9
22. Tselou E, Kiaris H (2008) Fibroblast independency in tumors: implications in cancer therapy. *Future Oncol* 4:427–32
23. Chu CY, Chang CC, Prakash E et al (2008) Connective tissue growth factor (CTGF) and cancer progression. *J Biomed Sci* 15:675–85
24. Aikawa T, Gunn J, Spong SM, Klaus SJ, Korc M (2006) Connective tissue growth factor-specific antibody attenuates tumor growth, metastasis, and angiogenesis in an orthotopic mouse model of pancreatic cancer. *Mol Cancer Ther* 5:1108–16
25. Cheng WF, Chang MC, Sun WZ et al (2008) Connective tissue growth factor linked to the E7 tumor antigen generates potent antitumor immune responses mediated by an antiapoptotic mechanism. *Gene Ther* 15:1007–16

26. Chen CA, Ho CM, Chang MC et al (2010) Metronomic chemotherapy enhances antitumor effects of cancer vaccine by depleting regulatory T lymphocytes and inhibiting tumor angiogenesis. *Mol Ther* 18:1233–43
27. Albini A, Tosetti F, Benelli R et al (2005) Tumor inflammatory angiogenesis and its chemoprevention. *Cancer Res* 65:10637–41
28. Benelli R, Morini M, Carrozzino F et al (2002) Neutrophils as a key cellular target for angiostatin: implications for regulation of angiogenesis and inflammation. *FASEB J* 16:267–9
29. Scapini P, Morini M, Tecchio C et al (2004) CXCL1/macrophage inflammatory protein-2-induced angiogenesis in vivo is mediated by neutrophil-derived vascular endothelial growth factor-A. *J Immunol* 172:5034–40
30. Calle EE, Rodriguez C, Walker-Thurmond K et al (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625–38
31. Calle EE, Thun MJ (2004) Obesity and cancer. *Oncogene* 23:6365–78
32. Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. *J Clin Invest* 115:1111–9
33. Hotamisligil GS, Arner P, Caro JF et al (1995) Increased adipose tissue expression of tumor necrosis factor- α in human obesity and insulin resistance. *J Clin Invest* 95:2409–15
34. Mohamed-Ali V, Goodrick S, Rawesh A et al (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *J Clin Endocrinol Metab* 82:4196–200
35. Wunderlich FT, Luedde T, Singer S et al (2008) Hepatic NF- κ B essential modulator deficiency prevents obesity-induced insulin resistance but synergizes with high-fat feeding in tumorigenesis. *Proc Natl Acad Sci U S A* 105:1297–302
36. Bromberg JF, Wrzeszczynska MH, Devgan G et al (1999) Stat3 as an oncogene. *Cell* 98:295–303
37. Khandekar MJ, Cohen P, Spiegelman BM (2011) Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer* 11:886–95
38. Barkan D, Green JE, Chambers AF (2010) Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. *Eur J Cancer* 46:1181–8
39. Ferrara N, Kerbel RS (2005) Angiogenesis as a therapeutic target. *Nature* 438:967–74
40. Wei YQ, Wang QR, Zhao X et al (2000) Immunotherapy of tumors with xenogeneic endothelial cells as a vaccine. *Nat Med* 6:1160–6
41. Chen XY, Zhang W, Zhang W et al (2006) Vaccination with viable human umbilical vein endothelial cells prevents metastatic tumors by attack on tumor vasculature with both cellular and humoral immunity. *Clin Cancer Res* 12:5834–40
42. Okaji Y, Tsuno NH, Kitayama J et al (2004) Vaccination with autologous endothelium inhibits angiogenesis and metastasis of colon cancer through autoimmunity. *Cancer Sci* 95:85–90
43. Scappaticci FA, Nolan GP (2003) Induction of anti-tumor immunity in mice using a syngeneic endothelial cell vaccine. *Anticancer Res* 23:1165–72
44. Yoshiura K, Nishishita T, Nakaoka T et al (2009) Inhibition of B16 melanoma growth and metastasis in C57BL mice by vaccination with a syngeneic endothelial cell line. *J Exp Clin Cancer Res* 28:13
45. Okaji Y, Tsuno NH, Tanaka M et al (2008) Pilot study of anti-angiogenic vaccine using fixed whole endothelium in patients with progressive malignancy after failure of conventional therapy. *Eur J Cancer* 44:383–90
46. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. *Nat Med* 9:669–76
47. Li Y, Wang MN, Li H et al (2002) Active immunization against the vascular endothelial growth factor receptor flk1 inhibits tumor angiogenesis and metastasis. *J Exp Med* 195:1575–84
48. Niethammer AG, Xiang R, Becker JC et al (2002) A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med* 8:1369–75
49. Zhou H, Luo Y, Mizutani M et al (2005) T cell-mediated suppression of angiogenesis results in tumor protective immunity. *Blood* 106:2026–32
50. Luo Y, Markowitz D, Xiang R et al (2007) FLK-1-based minigene vaccines induce T cell-mediated suppression of angiogenesis and tumor protective immunity in syngeneic BALB/c mice. *Vaccine* 25:1409–15
51. Liu JY, Wei YQ, Yang L et al (2003) Immunotherapy of tumors with vaccine based on quail homologous vascular endothelial growth factor receptor-2. *Blood* 102:1815–23
52. Xie K, Bai RZ, Wu Y et al (2009) Anti-tumor effects of a human VEGFR-2-based DNA vaccine in mouse models. *Genet Vaccines Ther* 7:10
53. Wei YQ, Huang MJ, Yang L et al (2001) Immunogene therapy of tumors with vaccine based on *Xenopus* homologous vascular endothelial growth factor as a model antigen. *Proc Natl Acad Sci U S A* 98:11545–50
54. He QM, Wei YQ, Tian L et al (2003) Inhibition of tumor growth with a vaccine based on xenogeneic homologous fibroblast growth factor receptor-1 in mice. *J Biol Chem* 278:21831–6
55. Luo Y, Wen YJ, Ding ZY et al (2006) Immunotherapy of tumors with protein vaccine based on chicken homologous Tie-2. *Clin Cancer Res* 12:1813–9
56. De Wever O, Nguyen QD, Van Hoorde L et al (2004) Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB J* 18:1016–8
57. Reardon DA, Akabani G, Coleman RE et al (2006) Salvage radio-immunotherapy with murine iodine-131-labeled antitenascin monoclonal antibody 81C6 for patients with recurrent primary and metastatic malignant brain tumors: phase II study results. *J Clin Oncol* 24:115–22
58. Scanlon MJ, Raj BK, Calvo B et al (1994) Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A* 91:5657–61
59. Cheng JD, Dunbrack RL Jr, Valianou M et al (2002) Promotion of tumor growth by murine fibroblast activation protein, a serine protease, in an animal model. *Cancer Res* 62:4767–72
60. Lee J, Fassnacht M, Nair S et al (2005) tumor immunotherapy targeting fibroblast activation protein, a product expressed in tumor-associated fibroblasts. *Cancer Res* 65:11156–63
61. Scott AM, Wiseman G, Welt S et al (2003) A Phase I dose-escalation study of sibtrotzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin Cancer Res* 9:1639–47
62. Loeffler M, Krüger JA, Niethammer AG et al (2006) Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J Clin Invest* 116:1955–62
63. Liao D, Luo Y, Markowitz D et al (2009) Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. *PLoS One* 4:e7965
64. Wen Y, Wang CT, Ma TT et al (2010) Immunotherapy targeting fibroblast activation protein inhibits tumor growth and increases survival in a murine colon cancer model. *Cancer Sci* 101:2325–32
65. Fassnacht M, Lee J, Milazzo C et al (2005) Induction of CD4(+) and CD8(+) T-cell responses to the human stromal antigen, fibroblast activation protein: implication for cancer immunotherapy. *Clin Cancer Res* 11:5566–71
66. Holbourn KP, Acharya KR, Perbal B (2008) The CCN family of proteins: structure-function relationships. *Trends Biochem Sci* 33:461–73
67. Robinson SC, Scott KA, Wilson JL et al (2003) A chemokine receptor antagonist inhibits experimental breast tumor growth. *Cancer Res* 63:8360–5

68. Rogers TL, Holen I (2011) Tumour macrophages as potential targets of bisphosphonates. *J Transl Med* 9:177
69. Luo Y, Zhou H, Krueger J et al (2006) Targeting tumor-associated macrophages as a novel strategy against breast cancer. *J Clin Invest* 116:2132–2141
70. Lewen S, Zhou H, Hu HD et al (2008) A Legumain-based minigene vaccine targets the tumor stroma and suppresses breast cancer growth and angiogenesis. *Canc Immunol Immunother* 57:507–15
71. Nozawa H, Chiu C, Hanahan D (2006) Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multi-stage carcinogenesis. *Proc Natl Acad Sci U S A* 103:12493–8
72. Heid HW, Moll R, Schwetlick I et al (1998) Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. *Cell Tissue Res* 294:309–21
73. Schmidt SM, Schag K, Müller MR et al (2004) Induction of adipophilin-specific cytotoxic T lymphocytes using a novel HLA-A2-binding peptide that mediates tumor cell lysis. *Cancer Res* 64:1164–70
74. Kessenbrock K, Plaks V, Werb Z (2010) Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 141:52–67
75. Page-McCaw A, Ewald AJ, Werb Z (2007) Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol* 8:221–33
76. Liotta LA, Tryggvason K, Garbisa S et al (1980) Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 284:67–8
77. Su JM, Wei YQ, Tian L et al (2003) Active immunogene therapy of cancer with vaccine on the basis of chicken homologous matrix metalloproteinase-2. *Cancer Res* 63:600–7
78. Yi T, Wei YQ, Tian L et al (2007) Humoral and cellular immunity induced by tumor cell vaccine based on the chicken xenogeneic homologous matrix metalloproteinase-2. *Canc Gene Ther* 14:158–64
79. Gassmann P, Enns A, Haier J (2004) Role of tumor cell adhesion and migration in organ-specific metastasis formation. *Onkologie* 27:577–82
80. White DE, Rayment JH, Muller WJ (2006) Addressing the role of cell adhesion in tumor cell dormancy. *Cell Cycle* 5:1756–9
81. Desgrosellier JS, Cheresh DA (2010) Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* 10:9–22
82. Lou YY, Wei YQ, Yang L et al (2002) Immunogene therapy of tumors with a vaccine based on the ligand-binding domain of chicken homologous integrin beta3. *Immunol Invest* 31:51–69
83. Oncology TL (2011) Tapping our immune potential to realise the ultimate goal. *Lancet Oncol* 12:1175
84. Finn OJ (2008) Cancer immunology. *N Engl J Med* 358:2704–15
85. Rosenberg SA, Restifo NP, Yang JC et al (2008) Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat Rev Cancer* 8:299–308
86. Mellman I, Coukos G, Dranoff G (2011) Cancer immunotherapy comes of age. *Nature* 480:480–9
87. Mitchell MS, Abrams J, Thompson JA et al (2007) Randomized trial of an allogeneic melanoma lysate vaccine with low-dose interferon Alfa-2b compared with high-dose interferon Alfa-2b for Resected stage III cutaneous melanoma. *J Clin Oncol* 25:2078–85
88. Rosenberg SA, Yang JC, Restifo NP (2004) Cancer immunotherapy: moving beyond current vaccines. *Nat Med* 10:909–15
89. Peng XC, Qiu M, Wei M et al (2012) Different combination schedules of gemcitabine with endostar affect antitumor efficacy. *Canc Chemother Pharmacol* 69:239–46