Targeting breast cancer cells and their microenvironment

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CHAPTER 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Breast cancer is the most common cause of cancer death among women worldwide (1). Recent treatment strategies focus on induction of tumor cell death using chemotherapeutic, anti-hormonal and targeted agents. However, it is increasingly recognized that not only the tumor cells, but also the tissue embedding the tumor cells; the tumor microenvironment, plays an important role in tumor progression. A role for the 'soil' was already suggested in 1890 by Paget who formulated this hypothesis based on the different metastasizing pattern between different primary tumors types (2). Accumulating data has confirmed this hypothesis and the role of the microenvironment has been included in the hallmarks of cancer (3). Also a gene expression signature based on breast cancer stromal tissue, was shown to be prognostic in different gene expression data sets (4). Interestingly, this signature identifies patients with worse outcome independently of breast cancer subtypes.

Also the influx of tumor infiltrating lymphocytes (TILs) is correlated with better patient outcome, which is breast cancer subtype dependent (5, 6).

The breast cancer microenvironment consists of several cellular components, soluble components and the extracellular matrix (ECM). An intense interplay between microenvironmental factors gives rise to a complex network, which modulates cancer behavior at various levels. Specific microenvironmental components induce pro- and anti-tumorigenic effects. The presence of tumor infiltrating TILs, for example, results in better patient outcome (5, 6). There is also a pro-tumorigenic aspect of the microenvironment (7), which is illustrated by the prognostic role of a higher relative amount of stroma in tumor tissue for primary breast cancer patients' outcome (8).

The effects of microenvironmental components on breast cancer behavior are manifold including tumor growth, migration and treatment sensitivity. Tumor growth is induced by soluble factors secreted by fibroblasts, macrophages and adipocytes (9-11). Indirectly, tumor growth is influenced by immune response modulation. Tumor growth is enhanced by the expression of membrane bound factors on cancer and immune cells resulting in immune response suppression, which leads to tumor growth (12). Tumor migration is enhanced by factors secreted by fibroblasts and adipocytes (13, 14). Integrins, metalloproteinases, lysyl oxidases secreted by cancer and stromal cells, guide migration of cancer cells by altering the ECM (15). Moreover, components of the microenvironment can affect treatment sensitivity. Efficacy of chemotherapy as well as targeted agents can be modulated by changing the stromal composition (16, 17). The composition of the microenvironment varies between tissue types, thereby giving rise to tissue dependent signals modulating breast cancer cell behavior (18).

Thus, the contributing role of the microenvironment to breast cancer behavior has become evident in the past years.

This thesis aims to further characterize the influence of the microenvironment on breast cancer behavior via different approaches and to describe strategies for exploiting the microenvironment for improved breast cancer treatment. With preclinical models, the functionality of the tumor-stroma interaction is studied and the effect of metastastic localization on tumor characteristics is studied in a clinical setting.

OUTLINE OF THE THESIS

In **chapter 2**, a systematic overview of relevant factors and processes in the breast cancer microenvironment is provided. Data was collected via PubMed, ClinicalTrials.gov and conference abstract databases of the San Antonio Breast Cancer Symposium and the latest annual meetings of the American Society Clinical Oncology and the American Association Cancer Research. We focus on the current knowledge of established processes in the breast cancer microenvironment and their clinical relevance. Of the key factors involved, the biological mechanisms, current strategies for intervention and prediction of treatment response are clarified. This overview aims to support optimizing (future) strategies for exploiting the microenvironment for improved breast cancer treatment.

Breast cancer research generally focuses on female breast cancer. Due to the rarity of the disease, male breast cancer specific data is scarce. As a result, men with breast cancer are treated according to therapy regimens optimized for female breast cancer patients. However, apparent differences exist between the male and female breast. The framework embedding the cancer cells is distinct and can thereby modulate breast cancer behavior gender specifically (19). To characterize the male breast cancer microenvironment, we perform an immunohistochemistry study based on cancer tissue of 803 male breast cancer patients. An important component of the breast cancer microenvironment comprises the interaction between cancer cells and immune system. **Chapter 3** focuses on immune factors in the male breast cancer microenvironment. The presence of TILs and the expression pattern of programmed death ligand (PD-L)1 and programmed death (PD)-1 in male breast cancer samples is evaluated by immunohistochemistry. Staining intensities are quantified and the relation with clinicopathological characteristics and patient survival is analyzed.

The role of the microenvironment in treatment sensitivity is studied in **chapter 4**. The bisphosphonate zoledronic acid shows an anti-cancer effect in breast cancer patients (20). A role for the microenvironment has been hypothesized in this setting, but is not confirmed. To study the role of the microenvironment with respect to zoledronic acid treatment, we optimized a tumor model of the chorioallantoic membrane (CAM) of fertilized chicken eggs. By using this *in vivo* model, the human stroma-breast cancer interaction can be studied with only limited interference of tissue of the host species. We compare the anti-cancer effect of zoledronic acid in the absence and presence of human stroma. The role of transforming growth factor (TGF)-β is

studied as mediator of the cancer-stroma interaction by quantification of protein expression and receptor activity with and without stromal cell presence.

Molecular imaging can provide local real time information about the *in vivo* interaction of the tumor and its microenvironment. In **chapter 5**, the feasibility of the *ex ovo* CAM assay of fertilized chicken eggs is explored for its use as preclinical *in vivo* imaging model. In this chapter is examined whether near infrared fluorescence labeled antibodies can be intravenously injected into the *ex ovo* CAM and subsequently quantified by measuring tracer uptake in breast cancer xenografts by IVIS imaging.

The microenvironment modulates breast cancer behavior in a tissue dependent fashion, which leads to organ specific metastases within one patient. Different metastatic surroundings could have implications for tumor characteristics and thus for therapy response. Currently, the most important molecular characteristic of breast cancer is estrogen receptor (ER)α. Overexpression of this receptor is present in approximately 75% of all breast cancers. However, limited knowledge is available on heterogeneity in ER expression across tumor lesions and their environment within metastatic breast cancer patients. 16α-[(18)F]-fluoro-17β-oestradiol ((18)F-FES) positron emission tomography (PET) can visualize the ER in tumor lesions, and tracer uptake is known to reflect ER expression. ^{18}F-FES-PET therefore can provide non-invasive whole body information on ^{18}F-FES tracer uptake. In **chapter 6**, existing ^{18}F-FES PET scans are re-evaluated for uptake in tumor lesions and tumor background of ER positive metastatic breast cancer patients to analyze ER heterogeneity in these patients. Cluster analysis was performed with different metastasis (imaging) features per patient as input variables.

Finally, in **chapter 7** the experimental results of this thesis are summarized which is followed by a discussion on the implications of our findings and an overview of future perspectives. **Chapter 8** provides a summary of the thesis in Dutch.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013 Available at http://globocan.iarc.fr
2. Paget S. The distribution of secondary growths in cancer of the breast. Lancet 1889;1:571–3.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
4. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 2008;14:518-27.
5. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 2013;31:860-7.
6. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259-71.
7. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423-37.
8. Gujam FJ, Edwards J, Mohammed ZM, Going JJ, McMillan DC. The relationship between the tumour stroma percentage, clinicopathological characteristics and outcome in patients with operable ductal breast cancer. Br J Cancer 2014;111:157-65.
9. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005;121:335-48.
10. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity 2014;41:49-61.
11. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nat Rev Cancer 2011;11:886-95.
12. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677-704.
13. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer--mechanisms underlying tumour progression and recurrence. Nat Rev Endocrinol 2014;10:455-65.
14. Tyan SW, Kuo WH, Huang CK, Pan CC, Shew JY, Chang KJ, et al. Breast cancer cells induce cancer-associated fibroblasts to secrete hepatocyte growth factor to enhance breast tumorigenesis. PLoS One 2011;6:e15313.
15. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009;139:891-906.
16. Domanska UM, Timmer-Bosscha H, Nagengast WB, Oude Munnink TH, Kruizinga RC, Ananias HJ, et al. CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. Neoplasia 2012;14:709-18.
17. Ostman A. The tumor microenvironment controls drug sensitivity. Nat Med 2012;18:1332-4.
18. Sevenich L, Bowman RL, Mason SD, Quail DF, Rapaport F, Elie BT, et al. Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. Nat Cell Biol 2014;16:876-88.
19. Ottini L. Male breast cancer: a rare disease that might uncover underlying pathways of breast cancer. Nat Rev Cancer 2014;14:643.
20. Valachis A, Polyzos NP, Coleman RE, Gnant M, Eidtmann H, Brufsky AM, et al. Adjuvant therapy with zoledronic acid in patients with breast cancer: a systematic review and meta-analysis. Oncologist 2013;18:353-61.
21. Schipper HS, de Jager W, van Dijk ME, Meerding J, Zelissen PM, Adan RA, et al. A multiplex immunoassay for human adipokine profiling. Clin Chem 2010;56:1320-8.
22. Rose DP, Vona-Davis L. Interaction between menopausal status and obesity in affecting breast cancer risk. Maturitas 2010;66:33-8.
23. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625-38.
24. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet 2014;384:755-65.