The complex interactions between the tumor microenvironment and prostate and oropharyngeal cancer

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CHAPTER 7

General Discussion

The tumor microenvironment (TME) is composed of multiple non-cancerous cells and soluble factors that play a pivotal role in the development and progression of many tumor types [1-3]. The composition of the TME largely depends on the anatomical site of the tumor, as well as environmental and genetic factors [4]. Moreover, the TME changes during tumor development [5]. Consequently, each tumor is unique and so is it's stroma.

During disease progression, immune cells and stromal cells are recruited into the TME where they may acquire an altered phenotype that supports survival and migration of cancer cells [6]. How the stromal cells change during cancer progression highly depends on the specific stimuli received from the surrounding TME [2]. Indeed, based on the composition of secreted cytokines and growth factors, the TME might become a crib or a threat for the tumor [7].

In this PhD thesis we described how different stromal cells of the TME have specific roles in the development of two very different cancer types, adenocarcinoma of the prostate (PCa) and oropharyngeal squamous cell carcinoma (OPSCC).

THE DUAL ROLE OF STROMAL AR IN PCA PROGRESSION

It was previously established that the Androgen Receptor (AR) is expressed in a variety of stromal cells. Cell-selective knockout models have been developed to improve the understanding of AR actions in a cell-specific manner [8]. Moreover, AR functions not only differ between different stromal cells, but also, opposite effects of stromal AR were described towards basal and luminal cell behavior in the normal prostate epithelium [9]. More specifically, stromal AR was shown to function as a promoter of PCa initiation in luminal epithelial cells, and as a suppressor of PCa initiation in basal epithelial cells [10]. Thus, these cell-specific AR functions might impact PCa initiation, development and the efficacy of anti-hormonal treatment of PCa patients in different ways.

Despite initial success of androgen deprivation therapy (ADT), eventually the treatment fails in virtually all patients, leading to recurrent tumor growth as castration-resistant prostate cancer (CRPC), which has high morbidity and mortality as hallmarks [11]. Since, AR is not only expressed in epithelial prostate cells, but also in cells of the TME, AR targeted therapy affects multiple cells. In order to understand how AR behaves in different cell types, tissue and cell-specific knock-out models have been developed, including tumor associated macrophages (TAM) and fibroblasts [8, 12-15]. However, the exact contribution of cell-specific AR signaling and the molecular mechanisms by which stromal AR might affect PCa development and progression remained unexplored.

In Chapter 3 and 4 of this thesis we described how the activation of AR signaling in different stromal cells, namely fibroblasts and macrophages, results in opposite effects on PCa progression. These findings showed how AR can exert cell-specific functions in different stromal cells of the prostate TME, with opposite effects on hormone therapy response. More specifically, in both fibroblasts and macrophages AR signaling was involved in regulation of the inflammatory response, however, AR signaling in fibroblasts was found to inhibit migration and invasion of PCa cells via downregulation of two potent chemokines, CCL2 and CXCL8. In contrast, AR

signaling in macrophages was found to support TAMs differentiation, leading to increased migration and invasion of PCa cells, via upregulation of the TREM-1-associated chemokines.

These data provided evidence for the dual role of AR in PCa stroma in prostate cancer progression, and stress the need for alternative therapies that act either by targeting AR in a cell specific manner, such as the selective androgen receptor modulators (SARMs) or by targeting the unwanted downstream effectors of AR signaling, including CCL2 and CXCL8.

THE HETEROGENEITY OF MACROPHAGE POPULATIONS IN PCA

Macrophages are extremely plastic cells which can express a wide range of surface and intracellular molecules that mediate distinct signaling pathways and functions, depending on the specific stimuli received from the surrounding TME,[16]. Since, macrophages are sensitive to external stimuli, the anatomical location as well as the pathophysiology of the tissue are key determinants of the polarization statuses of these cells [17]. Several studies explored the heterogeneity of macrophage populations *in vitro* and in mouse models [18-23]. These studies not only described previously unidentified macrophage populations, but also provided evidence for the presence of tissue-specific macrophage phenotypes, characterized by defined functions. Nevertheless, it is well known that, despite mice can mirror the human biology very well, there are significant differences in terms of cell surface and intracellular markers expression, especially for immunological markers [24, 25]. To date, very few studies explored the diversity of these cells in human cancer associated tissue at a single cell level [26].

Presently, the vast majority of markers used to identify TAMs in human cancer associated tissue are largely based on *in vitro* generated macrophages. More specifically, bone marrow-derived or monocyte-derived macrophages are stimulated *in vitro* with a variety of cytokines that lead to the differentiation into a large spectrum of different macrophage phenotypes, ranging from the pro-inflammatory to the anti-inflammatory macrophages [19, 20, 27]. However, given the extremely high plasticity of these cells, it is unlikely that the complex network of signals and molecules found in the tissue can be properly resembled in the binary cell culture system. Access to tissue-specific transcriptomic profiles of human macrophages would profoundly improve the predictive and prognostic role of macrophage in clinical studies.

In Chapter 5, we contributed to this knowledge by performing single cell transcriptomic analysis of human PCa resident macrophages and providing the first transcriptomic data of PCa-specific macrophage phenotypes. Three distinct populations were identified in the diseased prostate and from normal prostate tissue. However, no different transcriptional profiles were observed between macrophages isolated from the tumor-adjacent site of the prostatectomy specimen and those from the tumorous site, suggesting that tumorigenic factors also affect distant non-tumorigenic sites. Importantly, the three macrophage clusters were identified in all three patients included in the study, suggesting that our findings are widely generalizable. Consequently, immunotherapy treatment specifically targeting PCa associated macrophages, could potentially be effective in a large population of patients. However, to substantiate

this claim, this study should be repeated with more patients. The gene signatures generated from each macrophage cluster separately, was highly associated with both recurrence-free and metastasis-free survival. This association was not found with the canonical M1 and M2 macrophage phenotypes, highlighting the relevance of tissue-specific macrophage subtyping in the tumour microenvironment for prostate cancer progression. In conclusion, in this chapter, we demonstrated the utility of profiling single-cell transcriptomics in human tumor samples as a strategy to design gene classifiers for patient prognostication.

TAMS INFILTRATION AS A PROGNOSTIC MARKER IN OPSCC

In order to explore the role of the TME in squamous cell carcinoma development, the function of various TME components was studied in OPSCC. As shown in Chapter 6, TAMs infiltration in the OPSCC TME counteracts the protective role of HLA-II expression in tumor cells and negatively affects patients survival. More specifically, the number of TAMs was negatively correlated with expression of HLA-II on tumor cells in OPSCC patients, and patients with high TAM numbers and low tumoral HLA-II expression showed reduced disease-free survival. We speculated that IFN-g signaling could be a crucial modulator of this interaction as not only IFN-g promotes HLA-II expression, but also reduced the generation of TAMs [28, 29]. In agreement with the observed correlation between high number of TAMs and shorter survival, a large number of studies previously showed a correlation between TAMs infiltration in OPSCC and dedifferentiation processes, increased migration and growth of tumor cells [30, 31]. Moreover, a high number of infiltrated TAMs was correlated with clinical outcomes, including increased rate of disease relapses, occurrence of lymph node metastasis [32, 33] and suppression of the anti-tumor immunity [34, 35]. As a consequence, immunotherapy is currently used to target TAMs in different tumor types, including OPSCC. We believe that immunotherapy targeting TAMs would be particularly beneficial for OPSCC patients with high expression of HLA-II on tumor cells. Importantly, our study, highlighted the difference between HPV-positive and HPV-negative OPSCC, which is in agreement with numerous previous studies [36]. For more than a decade, we know that HPV-positive OPSCC have a distinct pathogenesis and clinical development. HPV positive OPSCC is induced by the HPV specific oncogenes E6 and E7, which is correlated with improved survival as compared to HPV-negative OPSCC. However, only recently, the composition of the TME was proposed as a major player in determining the faith of OPSCC patients. Indeed, the presence of viral HPV proteins often leads to increased infiltration of T lymphocytes into the OPSCC TME [37], which creates a pro-inflammatory microenvironment that promotes tumor killing [38]. In agreement with this, immunotherapy approaches were found to be more effective in HPV-positive OPSCC tumors compared to HPV-negative tumors, as HPV-positive are more profoundly infiltrated by immune cells [39].

In conclusion, our studies contributed to the knowledge of the mechanisms by which different TME components in both HPV-negative and HPV-positive OPSCC can be prognostic for patients' survival.

TARGETING TAMS IN CANCER PATIENTS TO IMPROVE TREATMENT RESPONSE

Many efforts have been made to understand the mechanism of reprogramming that drives macrophage polarization into pro-inflammatory and anti-tumor TAMs. As discussed previously, the TME is characterized by the preferential accumulation of M2-like pro-tumor macrophages that can strongly support tumor growth. New macrophage-targeting immunotherapies are aimed to convert M2-like macrophages into M1-like anti-tumor macrophages and with that suppressing cancer growth by increasing pro-inflammatory cytokines production [40]. One of the first attempts to re-polarize macrophage from M2-like to M1-like was by antibody-mediated inhibition of the IL-10 receptor and the CpG oligonucleotide-activating TLR9 receptor [41, 42], leading to increased activation of M1-like macrophages and increased Th1 cytokines production, which resulted in a significant therapeutic benefit in mice in both studies. Similarly, Specific re-education of macrophages was also accomplished by using CD40 agonists [43] and by using a dsRNA analog, PolyI:C, shown to induce inflammation and antitumor activity via TLR7/8 receptors [44] in mice. Furthermore, the role of the histidine-rich glycoprotein (HRG) in macrophage polarization was described in a hepatocellular carcinoma (HCC) mouse model [45]. HRG was shown to promote the M1-like macrophage polarization and inhibited tumor growth and metastasis, while improving chemotherapy efficacy. Finally, polarization of the macrophages from an immunosuppressive into a pro-inflammatory phenotype was also achieved by injecting mice with a combination of DNA vaccine directed against endoglin (CD105), a tumor vascular endothelial marker, in combination with interleukin-12-mediated therapy [46].

Alternative strategies to target the tumor-associated macrophages include targeting the CCL2-CCR2 axis to prevent monocytes/macrophages recruitment into the tissue, either by siRNA silencing of CCL2 [47], using anti-CCL2 antibodies [48, 49] or CCR2 inhibitors [50]. Also, inhibition of CSF1/CSF1R signaling was shown to inhibit TAMs proliferation [51-53] and induce reprogramming of TAMs into pro-inflammatory phenotypes [54]. Finally, several other means have been proposed to promote the conversion of TAMs into the M1-like phenotype, including Metformin, Sorafenib, Thymosin-a, Embelin, anti-MARCO antibody and inhibition of the NF-κB or STAT3 pathways [55, 56].

In conclusion, we believe that therapeutic approaches aimed to reprogram TAMs into pro-inflammatory and anti-tumor macrophages, rather than eliminating the entire macrophage spectrum should be exploited.

CONCLUSION AND FUTURE PERSPECTIVES

In this PhD thesis, we described the crucial role of the TME in the development of two distinct tumor types, adenocarcinoma of the prostate and oropharyngeal squamous cell carcinoma. As the composition of the TME is highly dependent on tissue-specific signals, there is a need to explore TME-tumor interactions in a cell-specific or tissue-specific manner. Ultimately, this would lead to the identification of molecular targets that might improve survival of patients

without systemic site-effects. Furthermore, the development of cell-specific therapies, including SARMs, would optimize the efficacy of antihormonal therapy.

In conclusion, our studies support that the characterization of the TME composition represents a strong predictor of treatment response in different types of tumors and that the TME bears multiple targets for future therapies.

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