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

Cioni, B.

CHAPTER 1

General introduction and outline

For many years, cancer has been described as the accumulation of germinal and somatic mutations of the genome, impairing the function of tumor suppressor genes and stimulating oncogenes [1]. Nowadays, it is commonly accepted that the tumor is not only a mass of malignant cells, rather than the result of a delicate network of interactions between tumor and stromal cells. Indeed, bidirectional communications between cancer cells and the surrounding microenvironment can strongly influence tumor development and progression [2]. Stromal cells might support tumorigenesis, either via direct cell-cell contact mechanisms with tumor cells, or by releasing specific factors, including cytokines and growth factors in the surrounding extracellular matrix (ECM), with remodeling of the tumor microenvironment (TME) as a result [3, 4].

The aim of this thesis is to elucidate the delicate network of interactions between different TME components and tumor cells in prostate cancer (PCa) and oropharyngeal squamous cell carcinoma (OPSCC).

THE YIN AND YANG OF THE TUMOR MICROENVIRONMENT

Immune cells are major components of the TME. They include members of the adaptive immunity such as T- and B- lymphocytes, as well as members of innate immunity, such as macrophages, dendritic cells (DCs) and natural killer (NK) cells [5]. Multiple studies previously explored the role of the immune composition in relation to development and progression of many tumor types [6-9], and it is nowadays well accepted the fact that characterization of the type, density and location of immune cells, have a prognostic value [10-12]. The degree of heterogeneity of immune cells and other stromal cells that accumulate in the TME depends on multiple factors, including stage of tumor development, anatomic location and external stimuli, such as cytokines and growth factors present in the TME [13-16]. Both anti-tumor and pro-tumor phenotypes of the same stromal cell type have been described in different tumors [17, 18]. Macrophages exert extreme plasticity and represent one of the most studied cells in terms of phenotypic diversity. The dual role of macrophages to promote or suppress tumor progression was introduced by Mantovani more than 15 years ago [19]. An oversimplified classification of the tumor-associated macrophage (TAM) phenotypes divides them into M1 or classically activated macrophages, usually associated with tumor regression, and M2, also called alternatively activated macrophages, usually associated with tumor progression [20]. However, great advances have been achieved in macrophage characterization in the last decade, in particular with the contribution of next-generation sequencing and the development of immunohistochemical methods. These studies have resulted in the identification of a large spectrum of macrophage polarization statuses, much broader than the M1 and M2 phenotypes, defined by specific functions and effects on tumor growth [21, 22]. Furthermore, it is now generally accepted the idea that a combination of TAMs markers, rather than individual markers can enhance the predictive power of TAMs infiltration in patients' outcome [23].

Similarly, cancer-associated fibroblasts (CAFs) can be distinguished from not cancer cell-associated or normal-associated fibroblasts (NAFs). While NAFs show inhibitory functions

on growth and cancer development via the release of inhibitory signals [24, 25], CAFs have been shown to possess promoting abilities for cancer cell growth, both in vitro and in vivo, and exhibit molecular and functional characteristics that might promote cancer progression via the secretion of specific growth factors, ECM proteins and immunomodulatory cytokines in the TME [26-28].

In summary, the specific composition of soluble factors found in the TME crucially modulates the phenotypic diversity of stromal cells. Moreover, in different organs, different soluble factors are released, and therefore, the role of the TME in cancer progression should be explored in a tissue specific manner.

PCA AND OPSCC AS MODELS OF COLD AND HOT TUMORS

Recent advances in cancer immunology and immunotherapy improved the understanding of the tissue-specific role of TME components in tumor progression and response to therapy [68]. Different cancer cell types colonize distinct organs, creating tissue specific tumor niches and secreting specific tumor-derived factors [69]. This suggests that anatomical location of the tumor dictates the TME composition and the specific sites where tumor cells tend to metastasize to. For instance, bones represent the primary metastatic site of prostate cancer cells, and their colonization of the bone is promoted by a repertoire of chemokines expressed in the stromal pre-metastatic niche, including CXCL12 and CXCL16 [29]. After colonization of the bone, specific factors secreted by PCa cells are able to promote osteoblastic lesions, ultimately leading to displacement of the bone marrow [30].

Tumors infiltrated by high numbers of immune cells, such as melanoma, lung cancer and oropharyngeal cancer are commonly called 'hot tumors', while poorly infiltrated tumors such as ER+ breast cancer and PCa, are called 'cold tumors' [31]. Hot tumors are generally associated with a high DNA mutational load and it is hypothesized that a high mutational load, will result into increased occurrence of neoantigens, which will enhance the recognition of the tumor as foreign. As a result, not only the number of infiltrating immune cells will increase but also their effectiveness in tumor killing [32, 33]. For this reason, hot tumors are considered good targets for immunotherapy treatment [34].

A large number of studies [35-37] focused on the predictive role of the tumor mutational burden after immunotherapy and enforced its role as a determinant of the immune mediated tumor killing and patient survival. However, cold tumors, generally characterized by low mutational burden, are the real challenge for immunotherapy. In fact, these tumors lack or show low numbers of infiltrating immune cells, especially T lymphocytes, which should exert tumor killing. However, while cold tumors often lack of a T lymphocyte population, they might harbor high numbers of immune suppressive myeloid cells, including TAMs and myeloid-derived suppressive cells (MDSCs), that sustain tumor growth [38], and will limit immunotherapy effectiveness.

In this PhD thesis, we explored the role of the TME in cancer progression in both a 'hot' and 'cold' tumor types: oropharyngeal squamous cell carcinoma and prostate cancer, respectively.

Oropharyngeal Squamous Cell Carcinoma

Oropharyngeal squamous cell carcinoma (OPSCC) is a type of head and neck cancer found in the soft palate, side and back wall of the throat, tonsil and 1/3 of the tongue. Tobacco and alcohol consumption are among the most important risk factors for OPSCC development [39]. In addition, less than 20 years ago human papilloma virus (HPV) infection was also identified as a risk factor of OPSCC [40]. More specifically, the HPV oncoproteins E6 and E7, were described to inactivate the tumor suppressor gene products p53 and Rb, respectively [41]. Frequency of HPV-positive OPSCC is highly variable depending on geographical areas, as the incidence of HPV infection changes based on sexual habits, tobacco and alcohol consumption rate. In Europe, it is estimated that 30-50% of the total cases of OPSCC are HPV-positive [42]. These tumors are more susceptible to radiation therapy and are associated with better overall survival (OS), longer progression-free survival (PFS) and lower metastasis rates [43-45] compared to HPV-negative tumors.

Similar to HPV-negative OPSCC patients, HPV-positive OPSCC patients are stratified in smokers and non-smokers. Patients with HPV-positive tumor who smoke have less favorable outcomes compared to non-smokers patients in the same group [46]. Importantly, accumulation of tobacco-derived mutations over time results in a reduced dependence of the tumor on the E6/E7 HPV -associated oncoproteins [47]. This suggests that tumors that arise with a HPV-induced molecular profile can develop tobacco-induced subclones over time, affecting prognosis and therapeutic decision making.

One of the possible explanations for the improved outcome observed in patients with HPV-infection is the viral-induced immune reaction that modulates the recruitment of specific immune infiltrates into the TME [48, 49]. Indeed, OPSCC is considered a 'hot tumor', characterized by high influx of immune cells, including T lymphocytes, macrophages and dendritic cells (DCs). Although, several studies explored the role of specific immune cells in relation to patients' survival [50-52], data elucidating the complex interactions between the different immune cell types in relation to HPV status are insufficient.

In this PhD thesis, we used OPSCC as a model of 'hot' tumor and studied the interactions between different immunological TME components and HPV-negative and HPV-positive OPSCC, and its relevance for patients' outcome.

Prostate Cancer

Prostate cancer (PCa) is the second most-common malignancy in men world-wide [53]. The vast majority of primary PCa are adenocarcinomas, and diagnoses is commonly preceded by a rise in serum prostate-specific antigen (PSA) levels. PCa confined to the prostate, can be treated with radiotherapy and radical prostatectomy with a curative intention [54]. However, approximately 35% of these patients will eventually develop a rise in PSA and a smaller

proportion will develop metastasis [55]. As PCa progresses, the TME architecture will change along, as a result of its dynamic nature. In fact, in each of the PCa developmental stages, from initiation to metastatic dissemination, the TME will have unique features [56]. In PCa, the TME show an altered phenotype which might support tumor growth, via increased ECM deposition and remodeling, increased protease activity, increased angiogenesis, and only in a minor part via influx of inflammatory cells. As already discussed, PCa is considered 'cold tumor', however, it is believed that the phenotype rather than the number of infiltrating immune cells have the biggest prognostic value in patients' outcome [57].

Androgen receptor (AR) is a nuclear steroid hormone receptor that plays a crucial role in PCa initiation and development, as it is vital for the proliferation, migration and apoptosis of tumor cells [58]. Impairment of AR signaling by hormone therapy is the most effective treatment for metastasized PCa patients [59], nevertheless, a large proportion of these patients will eventually develop castration resistant prostate cancer (CRPC). Androgens and AR signaling profoundly affect PCa TME composition as well, as AR it is also expressed in stromal cells, where it can modulate the release of specific cytokines and growth factors [60, 61].

In this PhD thesis, we have studied the molecular mechanisms of AR functions and consequences for PCa progression, in two of the most abundant stromal cell populations present in the PCa TME, namely fibroblasts and macrophages [62, 63].

CONCLUSIONS AND OUTLINE OF THE THESIS

In conclusion, the work described in this thesis aims to elucidate the critical role of the TME in modulating disease progression. Using two very different tumor types, we showed how stromal cells of the TME react to organ-specific stimuli to either promote or suppress tumor progression. With the recent advances in immunotherapy and the possibility to target specific stromal components and interfering in cell-specific signaling pathways, we now have the tools to enhance the efficacy of cancer treatments.

The studies presented in this PhD thesis, focus on the different interactions between tumor cells and the stromal cells, with a special interest in the phenotypic diversity of the myeloid cells.

Chapter 2 reviews the literature addressing the role of stromal AR in PCa development and progression. This chapter is focused on the potential role of AR in specific stromal cells and the consequences in PCa development and progression.

Chapter 3 reveals the role of AR signaling in CAFs and the effects on PCa progression. We propose a novel mechanisms by which AR signaling in CAFs results in a decreased production of CCL2 and CXCL8 chemokines, thus suppressing PCa cells migration and invasion in trans.

Chapter 4 presents the first genomic data on AR signaling in macrophages. This chapter shows the molecular mechanisms behind the AR-mediated expression of pro-tumorigenic cytokines by macrophages, that ultimately promote PCa cells migration and invasion. In addition, the role of AR in promoting the differentiation of macrophages into TAMs is elucidated. Chapters 3 and 4 highlight the dual role of stromal AR in PCa progression, as opposite effects have been observed in different stromal cell types.

Chapter 5 provides the first transcriptomic profile of native, PCa-associated macrophages at the single cell level. This chapter shows the degree of heterogeneity of macrophage populations in the PCa microenvironment. Specific macrophage phenotypes were identified with a significant impact in biochemical recurrence rate in PCa patients.

Chapter 6 presents a comprehensive multi-parametric study that explores the diversity of the TME composition in patients with HPV-negative and HPV-positive OPSCC. This study suggests that different phenotypes of T lymphocytes, macrophages and the expression of human leukocyte antigens (HLA) in the TME, affect survival of OPSCC patients.

Finally, **Chapter 7** summarizes the main results described in this thesis and provides personal interpretations and comments on the most relevant findings. It also contains possible clinical implications and suggests future directions.

REFERENCES

1. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
2. Joyce, J.A. and J.W. Pollard, *Microenvironmental regulation of metastasis.* Nat Rev Cancer, 2009. **9**(4): p. 239-52.
3. Barron, D.A. and D.R. Rowley, *The reactive stroma microenvironment and prostate cancer progression.* Endocr Relat Cancer, 2012. **19**(6): p. R187-204.
4. Mbeunkui, F. and D.J. Johann, Jr., *Cancer and the tumor microenvironment: a review of an essential relationship.* Cancer Chemother Pharmacol, 2009. **63**(4): p. 571-82.
5. Ferrone, S. and T.L. Whiteside, *Tumor microenvironment and immune escape.* Surg Oncol Clin N Am, 2007. **16**(4): p. 755-74, viii.
6. Takahashi, H., et al., *Dynamic changes in immune cell profile in head and neck squamous cell carcinoma: Immunomodulatory effects of chemotherapy.* Cancer Sci, 2016. **107**(8): p. 1065-71.
7. Zaynagetdinov, R., et al., *A critical role for macrophages in promotion of urethane-induced lung carcinogenesis.* J Immunol, 2011. **187**(11): p. 5703-11.
8. Takenaka, M., et al., *FOXP3 expression in tumor cells and tumor-infiltrating lymphocytes is associated with breast cancer prognosis.* Mol Clin Oncol, 2013. **1**(4): p. 625-632.
9. Bostrom, M.M., et al., *Tumor-Associated Macrophages Provide Significant Prognostic Information in Urothelial Bladder Cancer.* PLoS One, 2015. **10**(7): p. e0133552.
10. Pages, F., et al., *Effector memory T cells, early metastasis, and survival in colorectal cancer.* N Engl J Med, 2005. **353**(25): p. 2654-66.
11. Galon, J., et al., *Type, density, and location of immune cells within human colorectal tumors predict clinical outcome.* Science, 2006. **313**(5795): p. 1960-4.
12. Erdag, G., et al., *Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma.* Cancer Res, 2012. **72**(5): p. 1070-80.
13. Quail, D.F. and J.A. Joyce, *Microenvironmental regulation of tumor progression and metastasis.* Nat Med, 2013. **19**(11): p. 1423-37.
14. Devaud, C., et al., *Tissues in different anatomical sites can sculpt and vary the tumor microenvironment to affect responses to therapy.* Mol Ther, 2014. **22**(1): p. 18-27.
15. Huang, S.C.M., S.W. Tsao, and C.M. Tsang, *Interplay of Viral Infection, Host Cell Factors and Tumor Microenvironment in the Pathogenesis of Nasopharyngeal Carcinoma.* Cancers (Basel), 2018. **10**(4).
16. Balkwill, F.R., M. Capasso, and T. Hagemann, *The tumor microenvironment at a glance.* J Cell Sci, 2012. **125**(Pt 23): p. 5591-6.
17. Binnewies, M., et al., *Understanding the tumor immune microenvironment (TIME) for effective therapy.* Nat Med, 2018. **24**(5): p. 541-550.
18. Grivennikov, S.I., F.R. Greten, and M. Karin, *Immunity, inflammation, and cancer.* Cell, 2010. **140**(6): p. 883-99.
19. Mantovani, A., et al., *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends Immunol, 2002. **23**(11): p. 549-55.
20. Martinez, F.O. and S. Gordon, *The M1 and M2 paradigm of macrophage activation: time for reassessment.* F1000Prime Rep, 2014. **6**: p. 13.
21. Martinez, F.O., et al., *Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression.* J Immunol, 2006. **177**(10): p. 7303-11.
22. Mosser, D.M. and J.P. Edwards, *Exploring the full spectrum of macrophage activation.* Nat Rev Immunol, 2008. **8**(12): p. 958-69.
23. Heusinkveld, M. and S.H. van der Burg, *Identification and manipulation of tumor associated macrophages in human cancers.* J Transl Med, 2011. **9**: p. 216.
24. Sadlonova, A., et al., *Human breast fibroblasts inhibit growth of the MCF10AT xenograft model of proliferative breast disease.* Am J Pathol, 2007. **170**(3): p. 1064-76.
25. Hayashi, N. and G.R. Cunha, *Mesenchyme-induced changes in the neoplastic characteristics of the Dunning prostatic adenocarcinoma.* Cancer Res, 1991. **51**(18): p. 4924-30.
26. Sadlonova, A., et al., *Identification of molecular distinctions between normal breast-associated fibroblasts and breast cancer-associated fibroblasts.* Cancer Microenviron, 2009. **2**(1): p. 9-21.
27. Olumi, A.F., et al., *Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium.* Cancer Res, 1999. **59**(19): p. 5002-11.
28. Barclay, W.W., et al., *A system for studying epithelial-stromal interactions reveals distinct inductive abilities of stromal cells from benign prostatic hyperplasia and prostate cancer.* Endocrinology, 2005. **146**(1): p. 13-8.
29. Thobe, M.N., et al., *From prostate to bone: key players in prostate cancer bone metastasis.* Cancers (Basel), 2011. **3**(1): p. 478-93.
30. Obenauf, A.C. and J. Massague, *Surviving at a distance: organ specific metastasis.* Trends Cancer, 2015. **1**(1): p. 76-91.
31. Wargo, J.A., et al., *Monitoring immune responses in the tumor microenvironment.* Curr Opin Immunol, 2016. **41**: p. 23-31.
32. Lauss, M., et al., *Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma.* Nat Commun, 2017. **8**(1): p. 1738.
33. Riaz, N., et al., *Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab.* Cell, 2017. **171**(4): p. 934-949 e16.
34. Alexandrov, L.B., et al., *Signatures of mutational processes in human cancer.* Nature, 2013. **500**(7463): p. 415-21.
35. Hellmann, M.D., et al., *Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden.* N Engl J Med, 2018. **378**(22): p. 2093-2104.
36. Hellmann, M.D., et al., *Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer.* Cancer Cell, 2018. **33**(5): p. 843-852 e4.
37. Samstein, R.M., et al., *Tumor mutational load predicts survival after immunotherapy across multiple cancer types.* Nat Genet, 2019. **51**(2): p. 202-206.
38. Bonaventura, P., et al., *Cold Tumors: A Therapeutic Challenge for Immunotherapy.* Front Immunol, 2019. **10**: p. 168.
39. Elrefaey, S., et al., *HPV in oropharyngeal cancer: the basics to know in clinical practice.* Acta Otorhinolaryngol Ital, 2014. **34**(5): p. 299-309.
40. Gillison, M.L., et al., *Evidence for a causal association between human papillomavirus and a subset of head and neck cancers.* J Natl Cancer Inst, 2000. **92**(9): p. 709-20.
41. Yim, E.K. and J.S. Park, *The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis.* Cancer Res Treat, 2005. **37**(6): p. 319-24.
42. Anantharaman, D., et al., *Geographic heterogeneity in the prevalence of human papillomavirus in head and neck cancer.* Int J Cancer, 2017. **140**(9): p. 1968-1975.
43. Kimple, R.J., et al., *Enhanced radiation sensitivity in HPV-positive head and neck cancer.* Cancer Res, 2013. **73**(15): p. 4791-800.
44. Ang, K.K., et al., *Human papillomavirus and survival of patients with oropharyngeal cancer.* N Engl J Med, 2010. **363**(1): p. 24-35.
45. Molony, P., et al., *Impact of positive margins on outcomes of oropharyngeal squamous cell carcinoma according to p16 status.* Head Neck, 2017. **39**(8): p. 1680-1688.
46. Gillison, M.L., et al., *Tobacco smoking and increased risk of death and progression for patients with p16-*

positive and p16-negative oropharyngeal cancer. J Clin Oncol, 2012. **30**(17): p. 2102-11.

47. Zevallos, J.Y., E & Brennan, Paul & Liu, et al.,, *Molecular Profile of Human Papillomavirus–Positive Oropharyngeal Squamous Cell Carcinoma Stratified by Smoking Status.* International Journal of Radiation Oncology,Biology,Physics, 2016. **94**(4).

48. Oguejiofor, K., et al., *Distinct patterns of infiltrating CD8+ T cells in HPV+ and CD68 macrophages in HPV- oropharyngeal squamous cell carcinomas are associated with better clinical outcome but PD-L1 expression is not prognostic.* Oncotarget, 2017. **8**(9): p. 14416-14427.

49. Welters, M.J.P., et al., *Intratumoral HPV16-Specific T Cells Constitute a Type I-Oriented Tumor Microenvironment to Improve Survival in HPV16-Driven Oropharyngeal Cancer.* Clin Cancer Res, 2018. **24**(3): p. 634-647.

50. Ward, M.J., et al., *Tumour-infiltrating lymphocytes predict for outcome in HPV-positive oropharyngeal cancer.* Br J Cancer, 2014. **110**(2): p. 489-500.

51. Matlung, S.E., et al., *Differences in T-cell infiltrates and survival between HPV+ and HPV- oropharyngeal squamous cell carcinoma.* Future Sci OA, 2016. **2**(1): p. FSO88.

52. Seminerio, I., et al., *High infiltration of CD68+ macrophages is associated with poor prognoses of head and neck squamous cell carcinoma patients and is influenced by human papillomavirus.* Oncotarget, 2018. **9**(13): p. 11046-11059.

53. Ferlay, J., et al., *Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012.* Int J Cancer, 2015. **136**(5): p. E359-86.

54. Heidenreich, A., et al., *EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease.* Eur Urol, 2011. **59**(1): p. 61-71.

55. Murata, Y., et al., *Predictive factors of biochemical recurrence after radical prostatectomy for high-risk prostate cancer.* Int J Urol, 2018. **25**(3): p. 284-289.

56. Johansson, J.E., et al., *Fifteen-year survival in prostate cancer. A prospective, population-based study in Sweden.* JAMA, 1997. **277**(6): p. 467-71.

57. Barnes, T.A. and E. Amir, *HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer.* Br J Cancer, 2017. **117**(4): p. 451-460.

58. Heinlein, C.A. and C. Chang, *Androgen receptor in prostate cancer.* Endocr Rev, 2004. **25**(2): p. 276-308.

59. Labrie, F., *Blockade of testicular and adrenal androgens in prostate cancer treatment.* Nat Rev Urol, 2011. **8**(2): p. 73-85.

60. Cioni, B., W. Zwart, and A.M. Bergman, *Androgen receptor moonlighting in the prostate cancer microenvironment.* Endocr Relat Cancer, 2018. **25**(6): p. R331-R349.

61. Cioni, B., et al., *Loss of Androgen Receptor Signaling in Prostate Cancer-Associated Fibroblasts (CAFs) Promotes CCL2 and CXCL8 Mediated Cancer Cell Migration.* Mol Oncol, 2018.

62. Hayward, S.W., M.A. Rosen, and G.R. Cunha, *Stromal-epithelial interactions in the normal and neoplastic prostate.* Br J Urol, 1997. **79 Suppl 2**: p. 18-26.

63. Kruslin, B., M. Ulamec, and D. Tomas, *Prostate cancer stroma: an important factor in cancer growth and progression.* Bosn J Basic Med Sci, 2015. **15**(2): p. 1-8.