Thesis Summary

Functional analysis of pollutant nicotine-*CHRNA1* and identification of prognosis-related genes in melanoma using zebrafish and *in silico* approaches

(ゼブラフィッシュとインシリコ系を用いた、メラノーマにおけるニコチン - ニコチン性アセチルコリン受容体 A1 の機能解析と予後関連遺伝子の同定)

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During the last decade, targeted therapy and immunotherapies improved the survival rate of patients with melanoma. However, most patients acquire therapy resistance and disease relapse, especially in patients with progressed distant melanoma, whose 5-year survival rate is only 30%. A better understanding of melanoma progression and a complete characterization of the biological behavior of melanoma cells would help identify novel prognostic and therapeutic targets. In this thesis, we utilized *in vitro*, *in vivo*, and *in silico* approaches to investigate melanoma progression and prognosis. In chapter 2, we investigated the function of the nicotinic acetylcholine receptors (CHRNs) in melanoma, considering recent findings which imply that CHRNs stimulate cancer cell proliferation, progression, and invasion. Our analysis of the CHRNs revealed that *CHRNA1* was highly expressed in metastatic melanoma patients, zebrafish invasive melanoma cells, and metastatic melanoma cell lines treated with transforming growth factor-β1 (TGF-β1). Bioinformatics analysis for bulk RNA-sequencing (bulk RNA-seq) data of melanoma patients revealed that *CHRNA1* was significantly correlated with *CHRNB1* and *CHRNG* in the metastatic melanoma samples, and these muscle-type CHRNs were correlated with *ZEB1* and Rho/ROCK pathway-related genes. These findings suggest the involvement of the muscle-type CHRNs, especially *CHRNA1*, in melanoma progression and metastasis. We found that genes correlated to *CHRNA1* and single melanoma cells expressing *CHRNA1* were enriched with myogenesis, muscle contraction, and cell proliferation signatures. Furthermore, the *CHRNA1*-correlated genes and the myogenesis signature were correlated with the prognosis of melanoma patients. In chapter 3, we focused on the prognosis analysis of melanoma to identify prognostic signatures that can be used later in clinical applications. In this inspection, we used the advantage of integrating bulk RNA-seq and single-cell RNA-sequencing (scRNA-seq) bioinformatics analyses, including statistical and machine learning approaches. Our analysis revealed six distinctive gene expression signatures (GESs) exhibited by melanoma cells at the single-cell level. Deconvolution and

overall survival analyses showed that “Melanogenesis”, “Ribosomal biogenesis”, and “Extracellular structure organization” GESs and the “Immune cell interactions” GES correlated with poor and improved prognosis, respectively. We uncovered and validated a potential melanoma prognostic signature (referred to as MPS_45) composed of 45 genes enriched mainly with ribosomal and cell cycle-related genes. The identified MPS_45 showed a prognosis significance (*p*-value) better than or comparable to previous reports. In conclusion, we tried to tackle the melanoma progression and prognosis issues using *in silico*, *in vitro*, and *in vivo* approaches. On the hand of melanoma progression: we reported for the first time the expression and the involvement of the muscle-type CHRNs in melanoma progression. On the other hand, regarding melanoma prognosis: we used the GESs classification of melanoma cells at the single-cell level and constructed a potential prognosis-related gene signature for melanoma.