Summary

It is well known that cancer stem cells (CSCs) play a pivotal role in tumor metastasis, recurrence, heterogeneity, and resistance to chemotherapy and radiotherapy with poor prognosis in various cancers [1-5]. Therefore, CSCs are promising targets for cancer treatment, which when effectively executed could ultimately improve patient prognosis [5, 6]. The work described in this thesis examined the involvement of CSCs and several underlying regulatory mechanisms in both cancer development, and maintenance and acquisition of cancer stemness, as defined by tumor cell dissemination and metastatic potential, as well as stem cell properties in cancer models. Chapter 1 provides a general introduction on CSCs, related regulatory genes, current challenges, as well as the aim and outline of this thesis. Metastasis-associated protein 3 (MTA3), a tumor suppressor for many cancer types [7-9], has been suggested to mediate epigenetic remodeling of chromatin and may be involved in the regulation of cancer stemness [10]. However, the role of MTA3 in tongue squamous cell cancer (TSCC) and esophageal squamous cell carcinoma (ESCC) remains unclear. In Chapter 2, we first established TSCC cells with a shRNA knockdown or stably overexpression of MTA3 or SOX2 (a transcription factor essential for self-renewal of stem cells) alone or in combination and observed that the MTA3 inhibited SOX2. Next, we found that SOX2 played an indispensable role in the MTA3-mediated suppression of CSCs’ properties. Subsequently, we used 4-nitroquinoline 1-oxide (4NQO), a commonly used chemical carcinogen, to induce tumorigenesis in the mouse tongue mimicking human TSCC. Using this model, we showed that the levels of MTA3 decreased and SOX2 increased during the process of tumorigenesis and progression. Finally, we showed that the patients with MTA3low/SOX2high tumor expression had a worse prognosis, compared to those with any other expression pattern (MTA3high/SOX2high, MTA3high/SOX2low, MTA3low/SOX2low). This suggests that MTA3low/SOX2high might serve as an independent prognostic factor for TSCC patients. Altogether, our data suggest that MTA3 can inhibit TSCC CSC properties and tumor growth via downregulation of SOX2 and that MTA3low/SOX2high tumor expression might serve as a potential prognostic factor. Similarly, in Chapter 3, we demonstrate that also in ESCC MTA3 can repress both CSCs’ properties and metastasis in vitro and in vivo. Mechanistically, in ESCC MTA3 downregulates SOX2OT (SOX2 overlapping transcript, a long non-coding RNA crucial for regulating pluripotency) by forming an inhibitory complex with GATA3, subsequently inhibiting the SOX2OT/SOX2 axis, and ultimately inhibiting CSCs’ properties and metastasis. Depletion and overexpression of MTA3 in ESCC cells promoted and repressed 193 CSCs’ properties and metastasis, respectively. Furthermore, we demonstrated that MTA3 can repress SOX2OT/SOX2 axis by directly occupying the SOX2OT promoter region in ESCC cells. Interestingly, analysis of ESCC patient cohorts revealed that MTA3 levels are inversely correlated with SOX2OT, SOX2, tumor depth and advanced clinical stages. Moreover, patients with MTA3low/SOX2high expressing tumors showed a worse prognosis, compared to any other expression patterns (MTA3high/SOX2high, MTA3high/SOX2low, MTA3low/SOX2low), suggesting that MTA3low/SOX2high tumor expression could serve as an independent prognostic factor. Altogether, these results indicate that MTA3/SOX2OT/SOX2 axis plays a key role in not only CSCs’ properties but also clinical outcomes in ESCC. Therefore, this axis could be potentially used in cancer stratification and serve as a therapeutic target. Smoking is one of the most impactful risk factors for lifestyle-related cancers including ESCC [11-13]. Nicotine as a major component of tobacco and e-cigarettes has been reported not only to be responsible for addiction to smoking but also to promote migration and invasion of cells in vitro, as well as promote tumor growth and metastasis in vivo. This suggests that nicotine can enhance CSCs’
characteristics of the tumor, although mechanisms are far from clear [12, 14, 15]. In Chapter 4, we report that nicotine enhances CSCs’ properties by interacting with the cholinergic receptor nicotinic alpha 7 subunit (CHRNA7) thereby activating the JAK2/STAT3 signaling pathway in ESCC. Analysis of ESCC patient cohorts showed that aberrant CHRNA7 expression can act as an independent prognostic factor for ESCC patients. In multiple ESCC mouse models, dextromethorphan and metformin synergistically not only repressed nicotine-enhanced CSCs’ characteristics but also inhibited ESCC progression. Mechanistically, dextromethorphan inhibited CHRNA7 via non-competitively inhibited nicotine binding to CHRNA7 while metformin inhibited CHRNA7 expression by antagonizing nicotine-induced promoter DNA hypomethylation of CHRNA7. Since they are both FDA-approved drugs, the combination of these drugs may target nicotine-promoted CSCs in ESCC. Although previous results indicate that A-PaschiRNA can promote lymph node metastasis and enhance CSCs’ properties in ESCC [16], we explore the functions of A-PaschiRNA using a newly establish mouse model conditionally expressing A-PaschiRNA in Chapter 5. Mice carrying the A-PaschiRNA knockin gene do not display any apparent abnormalities, such as growth, fertility, histological, hematopoietic, and biochemical indices. Using this model, we explored the role of A-PaschiRNA in 4NQO-induced carcinogenesis of ESCC and found that A-PaschiRNA is capable of not only enhancing 4NQO-induced tumorigenesis but also increasing the aggressiveness of the cancer cells. In the future, the model could also be used to explore the roles of A-PaschiRNA in pathogenesis and potential targeted therapies. 194 Our previously study identified CD44+/CD24- , a subpopulation of esophageal cancer cells showing CSCs’ characteristics, reside in hypoxic niches in the tumors [17]. In Chapter 6, we aim to clarify the role of the hypoxia responding mammalian target of rapamycin (mTOR) pathway in esophageal CSCs. Our data showed that Torin-1, an mTOR inhibitor, upregulated SOX2, subsequently increasing the percentage of CD44+/CD24- , sphere formation potential, and autophagic activity. In contrast, MHY1485, an mTOR activator, induced the opposite effects. Moreover, Torin-1-mediated CSCs upregulation was significantly reduced in cells treated with autophagy inhibitor, hydroxychloroquine (HQC). Finally, using EC patient-derived organoids (ec-PDOs), a clearly defined CD44+/CD24- CSC population was detected, which was reduced using MHY1485. These results suggest that autophagy may play a key role in mTOR-mediated CSCs repression. Stimulation of the mTOR pathway might eliminate esophageal CSCs and therefore serve as a potential therapeutic target. In Chapters 2-6, we described some CSCs-related pathways including MTA3, ChRNA7, A-PaschiRNA, and mTOR, as well as underlying regulatory mechanisms in both cancer stemness and cancer development. Targeting these pathways using drugs that are currently existing or that will be developed could serve as a method to eliminate CSCs. Since accumulating evidence has showed CSCs can result in resistance to treatment, we next explored the potential of CSCs to predict patient response using CSCs-originating 3D cultured patient-derived organoids (PDOs). Several studies have indicated that PDOs may predict drug responses in the clinic, but their ability to predict responses to chemoradiation in esophageal cancer patients is still unknown. In Chapter 7, we generated organoids from patients with esophageal adenocarcinoma (ec-PDOs). The ec-PDOs can be cultured for up to 10 passages, indicating that ec-PDOs have the potential for self-renewal and expansion. Furthermore, the ec-PDOs stained positive for the EAC marker MUC5AC, suggesting an EAC origin. In response to irradiation, carboplatin, and paclitaxel, divergent response curves were observed in different ec-PDOs, while a similar response curve was seen in the same ec-PDOs during different passages. The results indicated that ec-PDOs exhibit interpatient response heterogeneity and the response to treatment can be assessed reproducibly. Moreover, after treatment with a combination of irradiation (IR), carboplatin (Car), and paclitaxel (Pac) to mimic EAC patients’ neoadjuvant chemoradiation, the ec-PDOs survival fractions from clinical complete response (cCR)
and partial response (PR) EAC patients are lower than that from progressive disease (PD) EAC patients. These preliminary findings suggest ec-PDOs might be used to predict patient-specific responses to clinical chemoradiotherapy for EAC patient