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Barrett's esophagus and esophageal adenocarcinoma: transcription factors and biomarkers

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Barrett's esophagus and esophageal adenocarcinoma: transcription factors and biomarkers

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Barrett's esophagus and esophageal adenocarcinoma: transcription factors and biomarkers

Thesis, University of Gronigen, Groningen, the Netherlands

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Preface

Preface

Esophageal cancer has a poor survival rate and its incidence is increasing. The 5-year survival of patients eligible for curative treatment is 50% (1), but late diagnosis in many patients decreases the overall 5-year survival to a mere 20% (2). Esophageal cancer is divided into two main histological categories: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Despite similar treatment options for both subtypes, EAC and ESCC differ considerably in etiology and tumor biology. ESCC occurs mostly in the proximal and middle part of the esophagus and is associated with smoking and alcohol consumption (3,4). EAC occurs preferentially in the distal part of the esophagus and persistent gastro-esophageal reflux and obesity (in particular abdominal obesity) are the main risk factors for EAC (5-7). Historically ESCC was more prevalent, but in the last decades the incidence of EAC showed a dramatic surge, especially in North-West Europe and North America, surpassing ESCC in incidence (8). The reasons for this increase are poorly understood, highlighting the need for a better understanding of EAC development.

The study of EAC carcinogenesis is greatly aided by the fact that EAC has a well-defined and easily accessible precursor lesion, Barrett's metaplasia of the esophagus (BE). BE is characterized by the presence of columnar epithelium in the distal end of the esophagus. Like EAC, persistent gastro-esophageal reflux disease (GERD) and obesity are the main risk factors for the development of BE (9). The prevalence of BE in the general population is estimated around 1,5% (10,11); since the prevalence of GERD is around 40% in the general population (11,12), only a fraction of patients with GERD develop BE. The clinical relevance of BE detection lies in its potential for malignant transformation through a metaplasia-dysplasia-adenocarcinoma sequence. Despite the fact that presence of BE is the most important risk factor for EAC development, a meta-analysis of BE surveillance studies suggests that BE patients only have a 0,3% risk of malignant transformation per patient year, but this increases to around 13% once dysplasia is present (9,13,14). While the absolute risk of BE malignant transformation is low, it is currently impossible to stratify BE patients according to the risk of malignant progression, and therefore all patients with BE are offered periodic endoscopic surveillance.

The current management of BE and EAC has been made possible by considerable progress that has been made in the understanding and clinical management of BE and EAC since the surgeon Norman Barrett first described BE in 1950 (1). The last decades acid-suppressant medical therapy has been introduced to control GERD. In addition, novel diagnostic and therapeutic endoscopic possibilities were developed, as well as new treatment regimens for EAC such as neoadjuvant chemoradiotherapy (nCRT). Despite these encouraging developments, several important challenges remain in the management of BE and EAC.

First, the current understanding of the molecular mechanisms underlying BE development is limited, and this hampers the development of effective pharmacological treatment modalities. Second, the current surveillance protocols are of limited efficacy, since the majority of EAC patients are not diagnosed at the stage of BE/early EAC, but instead present with advanced disease. Third, despite optimal treatment consisting of nCRT and surgery, the 5-year survival of EAC patients treated with curative intent is disappointing, and better insight into EAC biology is required to improve this. The studies presented in this thesis aim to address each of these three challenges and are discussed in more detail in the following section.

Scope of the thesis

Understanding Barrett's esophagus development

Aberrant activity of embryological signaling pathways has been implicated in the development and malignant progression of BE, but the relevant research data are fragmented across different disciplines of medicine and developmental biology. Therefore, in chapter 1 we provide a synthesis of the embryological signaling pathways and transcription factors involved in BE development and its malignant transformation, and offer a perspective on new therapeutic targets. Testing novel therapeutic targets requires representative pre-clinical models. Several approaches have been used to study BE and EAC including cell lines, animal and *in silico* models. Chapter 2 provides a discussion of the different models and the strengths and limitations of each, and offers suggestions for further development of novel pre-clinical models of BE and EAC.

One of the relevant embryological signaling pathways involved in BE development is the retinoic acid signaling pathway. Aberrant re-activation of the retinoic acid signaling pathway has been proposed to induce columnar differentiation of squamous epithelium (15), but the mechanisms through which retinoic acid exerts its effect on differentiation of squamous esophageal epithelium are poorly understood. In chapter 3 we studied the effect of retinoic acid on esophageal tissue morphology and differentiation in an organotypic *in vitro* model. The aim of chapter 4 was to study the expression of a panel of squamous and columnar transcription factors in normal squamous epithelium and BE *in vivo* and examine the effect of retinoic acid treatment on the expression of squamous and columnar transcription factors *in vitro*.

The transcription factor GATA6 is a known target gene of the retinoic acid signaling pathway. GATA6 expression contributes to the development of intestinal epithelium (16,17), but the expression pattern of GATA6 during BE development and malignant transformation is unknown. *GATA6* gene amplification was associated with a poor survival outcome in EAC patients (18), and the aim of chapter 5 was to study its expression in

different stages BE development and malignant transformation and determine the prognostic value of GATA6 protein expression in EAC.

Improving detection of Barrett's esophagus malignant transformation

The majority of patients that present with EAC were not previously included in BE surveillance programs, suggesting that most BE cases in the general population are not diagnosed (19). Early detection of premalignant esophageal lesions could drastically improve overall survival, but the absence of biomarkers indicative of metaplastic and/or malignant transformation in the esophagus hampers screening of populations at risk to develop BE and EAC. A surveillance strategy based on a blood-based biomarker seems an attractive approach, but so far no reliable circulating biomarkers have been identified. MiRNA's are small non-coding regulatory RNA species that are characterized by high tissue specificity and stability in serum. Tumor-specific serum miRNA profiles have been identified in various malignancies, including colon, lung, prostate and breast cancer (reviewed in (20)). In chapter 6 we set out to investigate the potential of circulating serum miRNA's as biomarkers of BE, dysplasia and EAC by comparing serum miRNA expression profiles from patients with BE, dysplasia and EAC with that of control patients.

Correlating esophageal adenocarcinoma survival with biomarker expression

Subpopulations of tumor cells with characteristics traditionally associated with stem cells (known as "Cancer Stem Cells", CSC), have an increased resistance to DNA damaging agents and increased metastatic potential (reviewed in (21)). Several proteins have been proposed as markers of CSC in various solid tumors, but little is known regarding the prognostic value of stemness-associated proteins in EAC. The aim of chapter 7 was to examine expression and prognostic value of a panel of potential CSC biomarkers in EAC patients undergoing primary surgical resection.

Neoadjuvant chemoradiotherapy (nCRT) reduces tumor volume and increases the chance of a complete tumor resection (1,22,23). Despite its success, further optimization of nCRT in EAC is hampered by the lack of predictive and prognostic biomarkers. Previous *in vitro* research suggested that increased activity of the Hedgehog signaling pathway was associated with resistance to chemoradiotherapy (24). In chapter 8 we studied the predictive and prognostic value of Sonic Hedgehog, a ligand of the Hedgehog pathway and the prognostic biomarkers identified in chapter 7 in a cohort of patients treated with both nCRT and surgery using pretreatment biopsies and post-treatment resection specimens.

Finally, chapter 9 provides a discussion of the data described in this thesis, and explores future perspectives regarding the opportunities to further improve BE and EAC management through a better understanding of the underlying biology.

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