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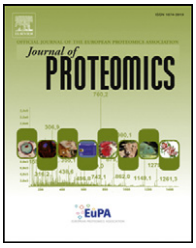

2D-DIGE analysis of sera from transgenic mouse models reveals novel candidate protein biomarkers for human gastric cancer

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ABSTRACT

The *gp130*$^{F/F}$ genetically engineered mouse (GEM) model reproducibly and predictably develops a gastric adenoma phenotype resembling the primary lesions of human intestinal-type gastric cancer (GC). Accordingly, changes to the serum proteome of *gp130*$^{F/F}$ mice may uncover early-stage GC biomarkers. Here, we have employed several double and compound mutant GEM strains that display distinct phenotypes with respect to gastric tumour load and inflammatory response, thereby mimicking different states of inflammation-associated early-stage GC in humans. This allowed us to distinguish between proteomic changes associated with tumourigenesis rather than confounding systemic inflammation. The comparative proteomic workflow involved depletion of high abundance proteins, 2D-DIGE analysis and protein identification by LC-MS/MS. The differential expression of 112 2D-DIGE spots specifically correlated with the tumour-bearing phenotype, corresponding to 31 murine proteins and their 28 human orthologues. Eight proteins were selected for validation in GC patient sera versus healthy controls. Significant increases in serum apolipoprotein E and haptoglobin, and decreases in afamin and clusterin, were confirmed by ELISA. Receiver operating characteristic analysis revealed that these proteins may be more sensitive and specific discriminators of GC than the existing clinical marker CA72-4.

Abbreviations: AACT, alpha-1-antichymotrypsin; apoE, apolipoprotein E; CA, carbohydrate antigen; CEA, carcinoembryonic antigen; FDR, false discovery rate; GC, gastric cancer; GEM, genetically engineered mouse; IGFALS, insulin like growth factor binding protein complex acid labile subunit; IL, interleukin; MARS, Multiple Affinity Removal System; PCA, principal component analysis; ROC, receiver operating characteristics; VDBP, vitamin D binding protein.

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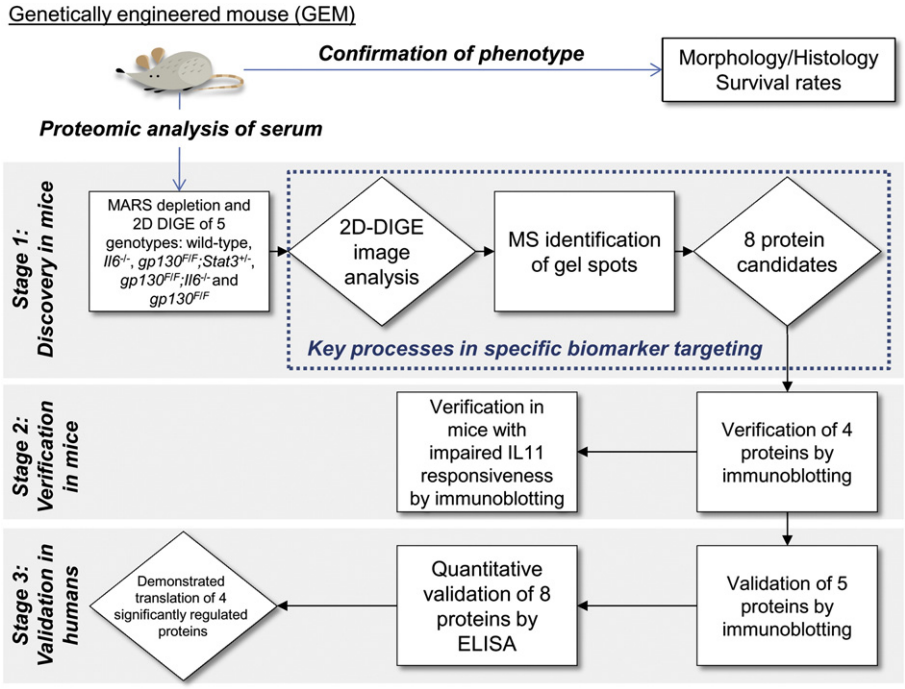

Fig. 1 – The overall workflow employed in this study. GEM of various genotypes underwent confirmation of phenotype based on morphology/histology and survival rates prior to proteomic analyses for the discovery, verification and validation of candidate serum protein biomarkers. Steps outlined with rectangular boxes were experimental processes while those represented by diamonds were analytical processes. The key steps involved in the identification and removal of those proteins regulated in association with confounding systemic inflammation rather than tumour development specifically are indicated.

1. Introduction

Gastric cancer (GC) is the fourth most common cancer in the world and the second leading cause of cancer mortality. In Western countries the expected five-year survival rate at diagnosis is <30% [1]. This can be attributed to a paucity of diagnostic symptoms associated with primary disease. Endoscopic examinations are frequently conducted at an advanced symptomatic stage when evidence of metastatic spread is present in >80% of patients [2]. The implementation of gastric photofluorography screening of asymptomatic individuals in Japan has increased the detection of early-stage GC and improved the overall five-year survival rate by >50% [3]. Although the sensitivity and specificity of gastric photofluorography are high [4], such programs show insufficient cost-effectiveness for Western countries where the incidence of GC is lower or the cost burdensome for less affluent countries such as those in South-East Asia and South America [5]. Accordingly, a non-invasive and cost-effective blood test based on reliable biomarkers would allow population-based screening to be combined with targeted follow-up of patients with endoscopy.

The serum biomarkers for GC currently used in the clinic, which encompass carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19–9 and CA72–4, are neither sufficiently sensitive nor specific for routine screening, and they are primarily used to monitor the progression of disease following treatment [6,7]. Meanwhile, the serum ratio of pepsinogen I/II has shown some promise as an alternative GC indicator in Asian populations, although its applicability in other racial groups, including Western populations, remains unclear [8,9]. Hence, a broader range of novel serum biomarkers suitable for detection of early-stage GC is highly desirable.

The discovery of serum biomarkers associated with early-stage (gastric) cancer is challenging for a number of reasons. These include the limited availability of samples from asymptomatic (early) GC patients, the genetic diversity of the human population combined with confounding co-morbidities, and the technical challenge in detecting anticipated low levels of biomarkers within the complex serum proteome [10]. Thus, mouse models recapitulating the molecular sequence of neoplastic progression in human cancers may provide alternative avenues for serum biomarker discovery. Genetically engineered mouse (GEM) models, where the development of spontaneous tumours is highly predictable and reproducible, add to the advantages afforded by genetic homogeneity of inbred strains that are reared under identical environmental conditions [11]. Importantly, the genetic composition of tumour-free control groups can be rigorously matched to facilitate assignment of observed differences in the serum proteome to tumour burden rather than confounding diseases.

Here, we take advantage of the extensively characterised homozygous *gp130*$^{F/F}$ GEM model, which spontaneously develops gastric adenomatous lesions in the glandular stomach [12]. We have previously shown that gastric lesions observed in *gp130*$^{F/F}$ mice, including those with additional mutations facilitating tumour development, recapitulate many of the progressive histological hallmarks associated with intestinal-type GC in humans [12–15]. This includes atrophic gastritis, intestinal metaplasia and epithelial dysplasia. Importantly, the gastric