TARGETING NANOPROBES FOR EARLY INVASIVE CANCER CELLS USING HEREDITARY DIFFUSE GASTRIC CANCER (HDGC) AS A MODEL.

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Introduction

The present work aims to improve early detection and disease prognosis in individuals at risk of hereditary diffuse gastric cancer (HDGC). For that purpose, we aim at identifying molecular markers specifically upregulated in invasive cancer cells, which will then be incorporated in nanoparticles designed to target diseased cells to improve disease diagnosis and prognosis.

The emerging field of Nanomedicine has been attracting unprecedented attention since nanostructures provide the means for monitoring cellular and molecular processes in vivo, in real time, with improved sensitivity and resolution. Nanoparticles can reach locations in the body of difficult access due to their reduced size and provide more convenient administration routes. Novel multifunctional nanoparticles can be engineered to target and diagnose a specific tissue or cell, to cross biological barriers, and be additionally combined with a pharmacological agent for applying therapy at early stages of the disease.

Improvements in the treatment of cancer have been slow mainly due to poorly predictable models, and lack of tumor targeting specificity and of effective cellular and intracellular delivery. Methods to increase local drug concentration at the tumor while lowering the systemic dose, coupled with the ability to kill only cancer cells while affecting as few healthy cells as possible, would result in more efficient treatments with fewer side effects..Hereditary Diffuse Gastric Cancer (HDGC) is a rare but devastating cancer susceptibility syndrome. It has been identified E-(epithelial) Cadherin germline mutations in families with HDGC, and pathology studies showed the presence of early invasive (intramucosal) diffuse carcinoma, without the presence of pre-malignant lesions, in all gastrectomy specimens performed to date in those patients. At the moment, prophylactic gastrectomy is still the best (and only) clinical approach, since endoscopic screening techniques are shown to be ineffective for surveillance of E-Cadherin mutant carriers. Therefore, the development of reliable screening methods for early detection, disease prognosis and continuous follow-up of these patients, is urgent.

Experimental

In a first approach we compared the expression profile, by cDNA array analysis, of two non-expressing E-Cadherin cell lines (MDA-MB 231 and MDA-MB 435) transduced with two E-Cadherin missense mutations (T340A and V832M) and with wild-type E-Cadherin, as control, in order to identify differentially expressed membrane proteins in mutant cells that could be used as molecular markers for primary diffuse gastric carcinoma. CD44 emerged as a differentially expressed protein in cells transduced with mutants of E-cadherin.

CD44 undergoes extensive alternative splicing, a process which is commonly dysregulated in neoplasic cells. CD44 gene contains ten variable exons (v1-v10) that can be spliced to generate (theoretically) hundreds of different protein isoforms, some of which have already been shown to be upregulated in neoplasia. If proved to be neoplastic-specific, some of these CD44 isoforms might constitute a potential biomarker as well as a target for therapeutic approaches.We selected a panel of six gastric cancer cell lines (NCI-N87, AGS, MKN45, MKN28, KATO-III and SNU-1) and one breast cancer cell line (MCF7), as well as normal gastric and breast tissue, for comparison. We amplified and cloned the entire variable region of the CD44 gene from all cell lines and normal tissues. We characterized all CD44 transcripts expressed by each one by sequencing. MKN28 cell line was used as a negative control since CD44 expression is silenced by promoter hypermethylation.SNU-1 cell line, also did not present any CD44 expression.

We observed that KATO-III and MKN45 E-Cadherin mutant gastric cancer cell lines harboured common aberrant CD44 transcripts which were absent in all other cell lines and normal tissues.

We hypothesize that this abnormal splicing profile could be used to target specific regions of the CD44 extracellular domain in E-Cadherin mutant cell lines, and to recognize tumor cells of diffuse gastric carcinoma with E-Cadherin mutations. In order to validate the neoplasic specificity of these CD44 transcripts further studies need to be performed.