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Loss of Anterior Gradient-2 expression is an independent prognostic factor in colorectal carcinomas

Marc-Oliver Riener [a,1], Thore Thiesler [b,1], Claus Hellerbrand [c], Thomas Amann [c], Gieri Cathomas [d], Florian Rudolph Fritzsche [e], Edgar Dahl [f], Marcus Bahra [g], Wilko Weichert [h,2], Luigi Terracciano [i,2], Glen Kristiansen [b,2,*]

[a] *Institute of Pathology, University Hospital Erlangen, Germany*
[b] *Institute of Pathology, University Hospital Bonn, Germany*
[c] *Department of Internal Medicine I, University Hospital Regensburg, Germany*
[d] *Cantonal Institute for Pathology, Cantonal Hospital Liestal, Switzerland*
[e] *Institute of Surgical Pathology, University Hospital Zurich, Switzerland*
[f] *Institute of Pathology, University Hospital Aachen, Germany*
[g] *Department of Visceral Surgery, Charité – Universitätsmedizin Berlin, Germany*
[h] *Institute of Pathology, University of Heidelberg, Germany*
[i] *Institute of Pathology, University Basel, Switzerland*

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Abstract ***Aims:*** The human Anterior Gradient-2 (AGR2) protein is strongly expressed in various human cancers, and it has been described to promote aggressive tumour features in some entities. So far, a comprehensive analysis of AGR2 expression in colorectal carcinomas has not been described.
Methods: Normal intestinal cells and colorectal carcinoma cell lines were analysed for AGR2 expression. AGR2 protein expression was immunohistochemically analysed in 28 normal tissue samples and 1068 tissue samples of clinically well characterised colorectal carcinomas. For statistical analysis, chi square test, spearman rank correlations, Kaplan–Meier estimates (Log rank test) and Cox regression were applied to test for diagnostic or prognostic associations.
Results: In the normal intestinal cell line and in normal colon mucosa AGR2 was found in all cases ($n = 28$). In contrast, loss of AGR2 was found in all six analysed colorectal cancer cell lines and in 833/1068 (78%) of the colorectal carcinoma tissue samples analysed, and it was significantly associated with a higher tumour grade and tumour localisation in the left-sided

* *Corresponding author:* Address: Institute of Pathology, University Hospital Bonn (UKB), Sigmund-Freud-Strasse 25, 53127 Bonn, Germany. Tel.: +49 228 287 15375; fax: +49 228 287 15030.
E-mail address: Glen.Kristiansen@ukb.uni-bonn.de (G. Kristiansen).
[1] MOR and TT share first authorship.
[2] Shared senior authors.

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colon. In addition to the conventional prognostic tumour parameters pT category, nodal status, metastasis and histological tumour grade the loss of AGR2 expression was significantly associated with reduced overall survival times in univariate and multivariate analyses, thus suggesting AGR2 as an independent prognostic factor in primary colorectal carcinoma.
Conclusions: AGR2 is frequently lost in colorectal carcinomas and might be a novel independent prognostic factor for overall patient survival.

1. Introduction

In the United States 142,570 estimated new cases of colorectal carcinoma (CRC) were expected for 2010 [1]. CRC ranks third of all new cancer cases in both sexes with respect to incidence and mortality [1]. Although this disease is potentially curable at early stages, the tumour frequently does not become symptomatic before advanced stages and then is associated with high mortality rates. Novel chemotherapeutic regimens combined with radiotherapy have considerably improved survival of patients with advanced tumours [2], however, novel therapeutical targets and prognostic factors to further intensify and individualise patient care are clearly needed. Conventional tumour parameters of the tumour-node-metastasis (TNM)-classification are indispensable prognostic markers, which are increasingly complemented by molecular markers. In this field our group has already made significant contributions by describing CD24 [3], ALCAM/CD166 [4], Polo-like kinase 1 [5] and histone deacetylases (HDACs) [6] as prognostic markers in patients with colorectal carcinoma.

The Anterior Gradient protein AGR2 (synonyms: hAG-2 [7], Gob-4 [8] is the human homologue to XAG-2 of *Xenopus laevis* [8]. AGR2 is located on chromosome 7p21. In oesophageal adenocarcinoma cell lines AGR2 promotes tumour growth, cell migration and cellular transformation [9]. The AGR2 protein has been described to be upregulated in prostate cancer, breast cancer and non-small cell lung cancer [10–12]. Several research groups found AGR2 expression in human breast cancer tissue and cell lines and its expression was associated with a positive oestrogen receptor status of the tumour cells [13,14]. cDNA microarray and additional immunohistochemical studies have identified AGR2 overexpression in pancreatic carcinomas [15–17]. Furthermore AGR2 was proposed for the detection of circulating tumour cells in the peripheral blood in patients with advanced cancers [18]. In the intestine AGR2 is essential for the production of mucus and mice lacking AGR2 were highly susceptible to colitis [19]. Further, these mice have decreased goblet cell Mucin 2, dramatic expansion of the Paneth cell compartment, abnormal Paneth cell localisation, elevated endoplasmic reticulum (ER) stress and severe terminal ileitis [20]. A cDNA study revealed that *AGR2* is down-regulated in the normal-adenoma-carcinoma sequence of colon carcinomas [21]. To our knowledge, a comprehensive AGR2 protein analysis in CRC has not yet been performed.

In order to clarify the expression patterns of AGR2 in colorectal carcinomas we studied its expression in colon carcinoma cell lines, normal colon mucosa and clinical samples from 1068 CRC patients and correlated our findings to clinical-pathologic parameters including overall survival times.

2. Materials and methods

2.1. Patients

In order to evaluate AGR2 in normal colon mucosa 28 resection specimens of patients with diverticulosis were analysed. Tissue samples from 122 patients from the Charité University Hospital Berlin, Germany and 457 patients from the Kantonsspital Liestal, Switzerland and 489 patients from the University Hospital Basel, Switzerland, who underwent colon/rectum resection were enclosed in this study (median age 72.0 years, range 15–100 years). Tissue microarrays were constructed as described previously [22]. Only patients with primary tumours and without other known malignancies at the time of diagnosis and at follow up were included. None of the patients received neoadjuvant therapy before surgery. Histological diagnosis was established on standard haematoxylin and eosin (H&E) stained sections of the respective tumours according to the guidelines of the World Health Organization (WHO). Clinical follow up data for 888 patients was available. The median follow up time of the patients was 42 months (range 1–153 months), 433 patients died during follow up (48.1%). The use of study materials has been approved by the respective local Ethics committees.

2.2. Cells and cell culture

Intestinal epithelial cells (IEC) were isolated as described [23,24]. Further, the colon carcinoma cell lines CaCo-2 (ATCC HTB-37), HT29 (ATCC HTB-38), SW48 (ATCC CCL-231), SW480 (ATCC CCL-228), HCT116 (ATCC CCL-247) and LoVo (ATCC CCL-229) were used. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin

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