Human Pathology (2016) **53**, 105–113

ELSEVIER

Human PATHOLOGY

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Original contribution

The expression profiles of the galectin gene family in colorectal adenocarcinomas☆,☆☆

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Received 18 December 2015; revised 14 February 2016; accepted 19 February 2016

Keywords:
Galectin;
Colorectal carcinoma;
Protein;
mRNA;
Immunohistochemistry

Summary We aim to investigate the expression profiles of galectin family genes (galectins-1, 2, 3, 4, 7, 8, 9, 10, and 11) in colorectal carcinomas. Messenger RNA (mRNA) expression of galectin family members (1, 2, 3, 4, 7, 8, 9, 10, and 12) was analyzed by real-time polymerase chain reaction in colorectal tissues from 201 patients (54 noncancer colorectal tissues, 49 adenomas, and 98 adenocarcinomas). Galectin-1 and galectin-3 protein expressions were determined by immunohistochemistry. In general, high galectin mRNA expression was noted in colorectal carcinomas in early stages of their pathogenesis. Significant differences in galectins-2, 3, 7, 8, and 10 mRNA expression were associated with pathologic stages ($P < .05$). Increased prevalence of galectins-2, 7, 8, and 10 mRNA overexpression was noted in nonmetastatic colorectal carcinomas ($P < .05$). Galectin-1 and galectin-3 proteins were present in the nucleus and cytoplasm of the colorectal tissues and expressed significantly higher in colorectal carcinomas when compared to colorectal adenomas (61% and 95%, respectively). Patients with colorectal carcinoma with high levels of galectin-3 mRNA and protein expression showed better prognosis ($P = .052$). To conclude, many novel correlations between the deregulation of galectin family genes and various clinicopathological features in colorectal adenocarcinoma were noted. Overexpression of galectins at the mRNA level and proteins were predominant in earlier stages of colorectal carcinomas. These altered expression patterns of galectin genes suggest the multifunctional role of galectin genes in the regulation of colorectal cancer development, progression, and metastasis.

1. Introduction

The galectins are a family of 15 mammalian galactoside binding proteins that share a consensus amino acid sequence in their carbohydrate recognition domains [1]. Altered expression of galectins has been implicated in cancer emergence

☆ Competing interests: There is no conflict of interest in publishing this article.
☆☆ Funding/Support: The project was supported by the project funding from Menzies Health Institute Queensland and joint funding of Griffith University and the Gold Coast University Hospital Foundation.

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http://dx.doi.org/10.1016/j.humpath.2016.02.016
0046-8177/

and progression [2]. Among the galectin family genes, galectin-1 and galectin-3 have been proposed to have a significant role in cancer pathogenesis, modulating the cell-to-cell or cell–to–extracellular matrix binding [3,4]. Galectin-3 plays key roles in cell adhesion, cell growth, and metastasis [5], whereas galectin-1 has been suggested to moderate the immune response and apoptosis [6]. In pathology practice, galectin-3 is a marker to help in the differentiation of papillary thyroid carcinoma from nonneoplastic thyroid lesions [7]. In colorectal cancer, galectin molecules have been reported to play a key role in the regulation of development, progression and metastasis of the cancer [8–9]. In this study, we aim to investigate the expression profiles of different galectin family genes (galectin-1, 2, 3, 4, 7, 8, 9, 10, and 11) in colorectal carcinomas with emphasis on galectin-1 and 3 protein expressions.

2. Materials and methods

2.1. Selection of patients

The patients chosen for this study had resection for primary colorectal adenomas (preinvasive neoplasia), colorectal adenocarcinomas, metastatic colorectal adenocarcinomas in distant organs, and nonneoplastic colorectal mucosae between 2005 and 2013 in Queensland, Australia. Patients were sequentially chosen and with no selection bias. Ethical approval of this study has been obtained from the Griffith University Human Research Ethics Committee (GU Ref no. MED/05/06/HREC).

Histologic sections were cut and stained for hematoxylin and eosin for microscopic examination. After histologic review, tissues from 201 patients (54 nonneoplastic mucosae, 49 adenomas, 98 primary adenocarcinomas) were recruited for this study. In addition, matched primary and metastatic tissue samples were collected. These included 26 lymph nodes with metastatic colorectal adenocarcinoma as well as 15 resected livers with metastatic colorectal adenocarcinoma. Tissue blocks were checked to ensure that they contained a representative cancer area.

2.2. Clinicopathological parameters

The colorectal adenocarcinomas were classified and graded and according to the World Health Organization criteria and staged according to TNM classification [10]. Clinical and demographic data collected include age, sex of the patients, and locations of the cancers. Pathologic parameters analyzed included size of the cancer, histologic grade, presence of metachronous/synchronous cancers, associated polyposis, cancer perforation, and lymphovascular invasion. Both conventional (n = 84/98) and mucinous adenocarcinomas (n = 14/98) (>50% mucin distribution in conventional adenocarcinomas) were included [11].

2.3. Clinical follow-up

The patients with colorectal adenocarcinoma were managed by a preagreed standardized protocol with the management of every patient being discussed in weekly multidisciplinary team meetings. Cancer recurrence or residual diseases after operation were recorded. The actuarial survival rate of the patients was calculated from the date of surgical resection of the colorectal cancers to the date of death or last follow-up.

2.4. RNA extraction and reverse transcription

Tissue blocks were sectioned into 7-μm slices for messenger RNA (mRNA) extraction. Preparation and mRNA extraction from these samples were performed as described previously [12]. Polymerase chain reaction (PCR) primers for galectin family members and controls are summarized in Table 1. A LightCycler 480 quantitative real-time PCR (Roche, Basel, Switzerland) was used to assess galectin family gene expression. All samples (unknown and standards) were run in triplicate and accompanied by a nontemplate control. Data analysis was performed as previously described [12]. A fold change of greater than 2 was considered as high expression, and a fold change expression of less than 1 was noted as low expression.

2.5. Tissue microarray

A tissue microarray (TMA) was constructed using a Model TMA Galileo CK3500 Tissue Microarrayer (Integrated System Engineering Srl, Milano, Italy). Briefly, all representative tumor donor blocks were cut for hematoxylin and eosin staining to define the morphology and pathology of the representative regions. From those regions, 3 cylindrical core tissue specimens (diameter, 0.6 mm) were arrayed into a recipient paraffin block (35 × 20 mm^2). Then, 4-μm sections were cut from the TMAs and processed for immunohistochemistry.

2.6. Immunohistochemistry

Immunohistochemistry for galectin-1 and galectin-3 proteins was performed manually using a high pH EnVision FLEX Mini Kit (Dako, Glostrup, Denmark). In brief, formalin-fixed, paraffin-embedded tissues were all sectioned to 4 μm. Then histologic slides were deparaffinized and rehydrated in xylene and ethanol. Slides were heated in epitope retrieval buffer (Dako) for 15 minutes using a microwave at low pressure.

For galectin-1, slides were incubated with galectin-1 monoclonal antibody (sc-166618) (Santa Cruz biotechnology, Dallas, TX) for 2 hours at room temperature. For galectin-3, a mouse monoclonal antibody was also recruited (sc-53127) (Santa Cruz Biotechnology). After a series of initial optimization steps, both primary antibodies for galectins-1 and 3 were diluted at 1:100 and incubated overnight at 4°C. The positive controls used were papillary thyroid carcinoma and colon