Experimental and Molecular Pathology 96 (2014) 212–218

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

The expression profiles of the galectin gene family in primary and metastatic papillary thyroid carcinoma with particular emphasis on galectin-1 and galectin-3 expression

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ARTICLE INFO

Article history:
Received 17 January 2014
Available online 12 February 2014

Keywords:
Galectin 1
Galectin 3
Galectin
Papillary thyroid carcinoma

ABSTRACT

Introduction: Galectin family members have been demonstrated to be abnormally expressed in cancer at the protein and mRNA level. This study investigated the levels of galectin proteins and mRNA expression in a large cohort of patients with papillary thyroid carcinoma and matched lymph node metastases with particular emphasis on galectin-1 and galectin-3.

Methods: mRNA expression of galectin family members (1, 2, 3, 4, 7, 8, 9, 10 and 12) were analysed by real-time polymerase chain reaction in 65 papillary thyroid carcinomas, 30 matched lymph nodes with metastatic papillary thyroid carcinoma and 5 non-cancer thyroid tissues. Galectin-1 and 3 protein expression was determined by immunohistochemistry in these samples.

Results: Significant expression differences in all tested galectin family members (1, 2, 3, 4, 7, 8, 9, 10 and 12) were noted for mRNA in papillary thyroid carcinomas, with and without lymph node metastasis. Galectin-1 protein was more strongly expressed than galectin-3 protein in papillary thyroid carcinoma. Galectin-1 protein was found to be overexpressed in 32% of primary papillary thyroid carcinomas. A majority of lymph nodes with metastatic papillary thyroid carcinoma (53%) had significantly increased expression of galectin-1 protein, as did 47% of primaries with metastases. *Galectin-1* mRNA levels were decreased in the vast majority (94%) of primary thyroid carcinomas that did not have metastases present. Galectin-3 protein levels were noted to be overexpressed in 15% of primary papillary thyroid carcinomas. In primary papillary thyroid carcinoma with lymph node metastases, 32% had over expression of galectin-3 protein. Overexpression of *galectin-3* mRNA was noted in 58% of papillary thyroid carcinomas and 64% of lymph nodes bearing metastatic papillary thyroid carcinoma. Also, primary papillary thyroid carcinoma with lymph node metastases had significantly higher expression of *galectin-3* mRNA compared to those without lymph node metastases.

Conclusion: Galectin family members show altered expression at the mRNA level in papillary thyroid cancers. Overexpression of galectin-1 and 3 proteins were noted in papillary thyroid carcinoma with lymph node metastases. The results presented here demonstrated that galectin-1 and galectin-3 expression have important roles in clinical progression of papillary thyroid carcinoma.

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Introduction

Galectins are animal lectins that have an affinity for β-galactose-containing oligosaccharides (Rabinovich, 2005). They may be located on the cell surface or be distributed in the cell cytoplasm and nucleus. The expression patterns of different galectins have been implicated in cancer emergence and progression (Balan et al., 2010). Of the galectin family, galectin-1 and galectin-3 have been most intensely studied in human cancers. Galectin-1 and galectin-3 have been proposed to be modulators of cell adhesion by mediation of cell-to-cell or cell-to-extra cellular matrix binding by cross-linking with glycoconjugates that contain β-galactosides (Chiariotti et al., 2004; Ito et al., 2012). Galectin-3 may also function as a modulator of cell growth through its influence on the cell cycle (Dumic et al., 2006), and galectin-1 has been suggested to moderate the immune response (Camby et al., 2006; Rabinovich, 2005). Due to their physiological roles, galectin-1 and 3 have been hypothesised to be involved in malignant transformation. Galectin-1 has been suggested to induce apoptosis of activated T-cells (Balan et al., 2010; Camby et al., 2006), whilst galectin-3 may act as an adhesion molecule in tumour progression (Cvejic et al., 2005a; Kawachi et al., 2000) and loosen the connection of tumour cells to promote metastasis (Kawachi et al., 2000).

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0014-4800/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexmp.2014.02.003

In thyroid cancer, the most common histological subtype is papillary thyroid carcinoma (Lam et al., 2005). Expression of galectin-3 is used as one of the markers to differentiate papillary thyroid carcinoma from benign thyroid nodules in pathology practice (Wu et al., 2013). Galectin-1 and 3 have been shown to be abnormally expressed in a range of tumour sub-types at the protein level (Beesley and McLaren, 2002; Brown et al., 2006; Cvejic et al., 2005a; Feilchenfeldt et al., 2003; Htwe et al., 2010; Inohara et al., 1999; Kawachi et al., 2000; Mehrotra et al., 2004; Orlandi et al., 1998; Saussez et al., 2008; Türköz et al., 2008; Xu et al., 1995) and at the mRNA level (Bernet et al., 2002; Feilchenfeldt et al., 2003; Takano et al., 2003). The exact expression profiles of galectin-1 and galectin-3 in thyroid carcinoma is not consistent in the literature, however. Galectin-1 protein, as well as galectin-3 protein and mRNA expression have been found to be increased in a wide variety of thyroid carcinoma types (Beesley and McLaren, 2002; Bernet et al., 2002; Brown et al., 2006; Cvejic et al., 2005a; Feilchenfeldt et al., 2003; Inohara et al., 1999; Kawachi et al., 2000; Mehrotra et al., 2004; Orlandi et al., 1998; Takano et al., 2003; Xu et al., 1995). Other studies have suggested a decrease in galectin-3 protein expression in metastatic carcinomas (Htwe et al., 2010; Türköz et al., 2008) and decrease in galectin-3 mRNA expression in certain thyroid tumour types (Feilchenfeldt et al., 2003). To resolve these inconsistencies in the literature, this study aimed to document the mRNA expression of major members of galectin family in thyroid carcinoma and then investigated the level of protein expression of the most studied members, galectin-1 and galectin-3, in thyroid carcinoma, with or without metastasis through the utilisation of a large patient cohort.

Materials and method

Materials

In total, 65 conventional papillary thyroid carcinomas, 30 lymph nodes with metastatic papillary thyroid carcinomas and 5 non-cancer thyroid tissues were selected and obtained from archival formalin-fixed, paraffin-embedded tissues recruited from different hospitals in Australia after full ethical approval was obtained. From each case, all the slides were examined and a block was selected for the study. The malignant thyroid tumours were classified with reference to the criteria defined by World Health Organization classification of malignant tumours (Weir et al., 2003). Only conventional papillary thyroid carcinoma was included in this study. Follicular variant and other variants of papillary thyroid carcinoma were excluded from the analysis. The American Joint Committee on Cancer (AJCC)/International Union against Cancer (UICC) tumour-node-metastasis (TNM) staging system was used to stage the thyroid tumours (Sobin and Wittekind, 2009). From the selected blocks, 4 μm thick histologic sections were cut and stained with haematoxylin and eosin and reviewed to confirm the histology and adequacy of the specimen before proceeding for further study.

RNA extraction and reverse transcription

Total RNA was isolated and extracted from archival tumour formalin fixed paraffin embedded tissue samples using Qiagen miRNeasy FFPE Kits (Qiagen Pty. Ltd., Hilden, NRW, Germany). The quality of RNA was assessed by the use of an Experion electrophoresis instrument (Bio-Rad, Hercules, CA, USA). RNA was converted to cDNA using miScript Reverse Transcription kit (Qiagen) according to the manufacturer's instructions. Preparation and evaluation of these samples has been described previously (Salajegheh et al., 2011).

Real-time polymerase chain reaction

Primers were designed for analysis of expression of *Galectin-1* (GenBank accession number NM_001001867), *Galectin-2* (*NM_006498.2*), *Galectin-3* (GenBank accession number NM_001102341), *Galectin-4* (GenBank accession number NM_006149.3), *Galectin-7* (GenBank accession number NM_002307.3), *Galectin-8* (GenBank accession number NM_006499.4), *Galectin-9* (GenBank accession number NM_009587.2), *Galectin-10* (GenBank accession number NM_001828.5), and *Galectin-12* (GenBank accession number NM_001142535.1). Ubiquitous controls for these experiments consisted of *GAPDH* (GenBank accession number NM_002046) and the 18S ribosomal subunit (GenBank accession number NR_003286.2). PCR primers for Galectin family members and controls are summarised in Table 1. RT-PCR was performed in a total volume of 20 μl reaction mixture containing 10 μL iQ SYBR green supermix (Bio-Rad), 1 μL of each 5 μmol/L primer, 6 μl of dH20, and 1 μL of sample cDNA (30 ng/μl). All samples (unknown and standards) were run in triplicate and accompanied by a non-template control. Thermal cycling conditions included initial denaturation in 1 cycle of 3 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C. Melting curve analysis was also performed using 81 cycles of 30 s increasing from 55 °C. The melting curves of all final real-time PCR products were analysed for determination of genuine products and contamination by non-specific products and primer dimers. For assessment, the mean values of differences in cycle thresholds (ΔCt) for each triplicate in the PCR were calculated, using the average of the ct values for both ubiquitous control genes. The expression of genes of interest was normalised in each sample using the ΔΔCt of the target gene and ubiquitous control genes in different cell subtypes. The fold changes in the target genes were calculated for each sample group using the $2^{-\Delta\Delta Ct}$ method, comparing to non-cancer tissue (Livak and Schmittgen, 2001). The fold changes <0.5 were considered as low expression, fold changes >0.5 and <2 were considered as normal expression, and fold changes >2 were considered as high expression.

Immunohistochemistry staining for galectin-1 and galectin-3

A tissue microarray (TMA) was constructed using a Model TMA Galileo CK3500 tissue Microarrayer (Integrated System Engineering Srl [ISE], Milano, Italy). Briefly, all representative tumour donor blocks were cut for haematoxylin & 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 acquired and arrayed into a new recipient paraffin block (35×20 mm^2). Then, sections of 4 μm were cut from the TMAs and processed for immunohistochemistry. Immunohistochemistry for galectin-1 and galectin-3 was performed in IntelliPATH FLX autostainer (Biocare Medical, Concord,

Table 1
PCR primers forward and reverse for galectin family members used in this study.

Galectin member	Product size (bp)		Sequence
Galectin-1	Product 84	Forward	TGCAACAGCAAGGACGGC
		Reverse	CACCTCTGCAACACTTCCA
Galectin-2	Product 94	Forward	GATGGCACTGATGGCTTTG
		Reverse	AGACAATGGTGGATTCGCT
Galectin-3	Product 108	Forward	CAGAATTGCTTTAGATTTCCAA
		Reverse	TTATCCAGCTTTGTATTGCAA
Galectin-4	Product 144	Forward	CGAGGAGAAGAAGATCACCC
		Reverse	CTCTGGAAGGCCGAGAGG
Galectin-7	Product 141	Forward	CAGCAAGGAGCAAGGCTC
		Reverse	AAGTGGTGGTACTGGGCG
Galectin-8	Product 95	Forward	CTTAGGCTGCCATTCGCT
		Reverse	AAGCTTTTGGCATTTGCA
Galectin-9	Product 91	Forward	CTTTCATCACCACCATTCTG
		Reverse	ATGTGGAACCTCTGAGCACTG
Galectin-10	Product 114	Forward	AGTGTGCTTTGGTCGTCGT
		Reverse	ATGCTCAGTTCAAATTCTTGG
Galectin-12	Product 111	Forward	TGTGAGCCTGAGGGACCA
		Reverse	GCTGAGATCAGTTTCTTCTGC
GAPDH	Product 87	Forward	TGCACCACCAACTGCTTAGC
		Reverse	GGCATGGACTGTGGTCATGAG
18S ribosome	Product 71	Forward	CTTAGAGGGACAAGTGGCG
		Reverse	GGACATCTAAGGGCATCACA

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