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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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 nodes 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.

Introduction

Galectins are animal lectins that have an affinity for β -galactose-containing oligosaccharides [1]. 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 [2]. 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 [3, 4]. Galectin-3 may also function as a modulator of cell growth through its influence on the cell cycle [5], and galectin-1 has been suggested to moderate the immune response [1, 6]. 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 [2, 6], whilst galectin-3 may act as an adhesion molecule in tumour progression [7, 8] and loosen the connection of tumour cells to promote metastasis [7].

In thyroid cancer, the most common histological subtype is papillary thyroid carcinoma [9]. Expression of galectin-3 is used as one of the markers to differentiate papillary thyroid carcinoma from benign thyroid nodules in pathology practice [10]. Galectin-1 and 3 have been shown to be abnormally expressed in a range of tumour sub-types at the protein level [7, 8, 11- 20] and at the mRNA level [13, 21, 22]. 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 [7, 8, 11-14, 16, 19-22]. Other studies have suggested a decrease in galectin-3 protein expression in metastatic carcinomas [17, 18] and decrease in galectin-3 mRNA expression in certain thyroid tumour types [13]. 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 [23]. 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 [24]. 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 [25].

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 summarized in Table 1. RT-PCR was performed in a total volume of 20µl reaction mixture containing 10µL iQ SYBR green supermix (Bio-Rad), 1 µL of each 5 µmol/L primer, 6 µl of dH₂O, and 1 µL of sample cDNA (30ng/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 minutes at 95°C, followed by 40 cycles of 10 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72°C. Melting curve analysis was also performed using eighty one cycles of 30 seconds 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 (ΔC_t) for each triplicate in the PCR were calculated, using the average of the C_t values for both ubiquitous control genes. The expression of genes of interest was normalized in each sample using the $\Delta\Delta C_t$ 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 $2^{-\Delta\Delta C_t}$ method, comparing to non-cancer tissue [26]. 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 x 20mm²). Then, sections of 4 μ m 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, CA, USA), providing more standardized results than manual staining techniques. In brief, formalin-fixed, paraffin-embedded tissues were all cut into 4 μ m. Then histological slides were deparaffinised and rehydrated in xylene and ethanol. Slides were heated in 0.01 M citrate buffer for 20 minutes at 80°C in Rapid Multifunctional Microwave Tissue Processor Model KOS (Milestone, Sorisole, Bergamo, Italy). After being allowed to cool to room temperature and washed in phosphate buffered saline (PBS), immunostaining was performed using the Biocare Medical Mach 4 polymer detection system (Biocare Medical).

For galectin-1, slides were incubated with galectin-1 antibody (sc-166618) mouse monoclonal IgG_{2a} by Santa Cruz (1:100, Santa Cruz biotechnology, California, U.S.A.) for 2 hours at room temperature. Galectin-3 Antibody (A3A12) is a mouse monoclonal IgG (sc-53127) by Santa Cruz biotechnology was used at a 1:100 dilution for 3 hours at room temperature for detection of galectin-3 in the population of the study. The positive controls

used were colon cancer tissue known to express galectin-1 and galectin-3 proteins. Omission of primary antibodies served as negative control.

Galectin-1 and galectin-3 cytoplasmic immunostaining intensity was examined and scored by the author (AKL) blinded to the RT-PCR results of the samples. The results were assessed semi-quantitatively and scored into three categories. Cases with no detectable immunostaining of carcinoma cells were scored zero. Tissue sections graded as 1 showed a weak staining of the majority of tumour cells. Cases with a moderate or strong staining intensity were scored as grade 2.

Data analysis

All RNA and protein expression data was entered into the statistical analysis software, Statistical Package for Social Sciences, SPSS version 22.0 (IBM SPSS Inc., New York, NY, USA). Final normalised data was analysed using Student's t-test and ANOVA using Bonferroni and LSD correction for continuous variables and chi-square or Fisher exact tests for categorical variables. In addition, Pearson correlation (2-tailed) test was used for correlation analysis. Significance level was taken at $p < 0.05$.

Results

Galectin family mRNA expressions

Decreased expression of *galectin-1* mRNA was found in 77% (n=50) of the primary papillary thyroid carcinomas ($p<0.05$), whilst a normal expression profile of *galectin-1* mRNA was detected in 63% (n=19) of lymph nodes where the cancer had metastasised ($p<0.05$). The primary papillary thyroid carcinoma group was then divided into two sub-groups, primary papillary thyroid carcinoma without lymph node metastasis and with lymph node metastasis. In comparison, 94% (n=30) of papillary thyroid carcinoma with negative lymph node samples whereas 60% (n=20) of papillary thyroid carcinoma with positive lymph node specimens had a decreased expression of *galectin-1* mRNA ($p<0.05$). Overexpression of *galectin-3* mRNA was noted in 58% (n=38) of all papillary thyroid carcinomas and 64% (n=19) of lymph node with thyroid cancer metastases lesions. Also, papillary thyroid carcinoma with lymph node metastases had significantly higher expression of *galectin-3* mRNA expression compared to those without lymph node metastases (70% versus 47%, $p=0.009$). Furthermore, no correlation between clinicopathological features of papillary thyroid carcinoma and *galectin-1* and *galectin-3* mRNA expressions were noted.

Significant expression differences for *galectins 2, 4, 7, 8, 9, 10* and *12* mRNA were all noted amongst different subcategories of papillary thyroid carcinomas, lymph node metastases and non-cancer control tissues ($p<0.05$). *Galectin-3* and *10* in papillary thyroid carcinomas with lymph node metastasis were significantly increased compared to non-metastatic papillary thyroid carcinomas ($p<0.05$) (Figure 1). On the other hand, a significant reduction in expression of *galectin-7* was noticed in those primary metastatic papillary thyroid carcinomas when compared to non-metastatic papillary thyroid carcinoma, though lymph nodes themselves showed significantly higher expression than metastatic primaries or control tissue ($p<0.05$) (table 2). *Galectin-2, 8, 9* and *10* showed a trend of increasing

expression as a tissue became more malignant and metastatic, with *galectin-2*, 8 and 9 all examples showing significant increases in expression compared to normal tissues in metastatic lymph nodes. *Galectin-4* showed significantly increased expression in metastatic lymph nodes compared to metastatic and non-metastatic primary tumours, but not control tissues. *Galectin-12* on the other hand, showed universal significant decreases in expression in all categories of cancer tissue compared to normal thyroids.

There were a significant correlation between T staging of papillary thyroid carcinomas of this study and the mRNA expression levels of *galectin-4* and *12*. *Galectin-4* and *galectin-12* were significantly underexpressed in cancer with lower T stages (T1 and T2 combined) ($p=0.037$ for *galectin-4* and $p=0.042$ for *galectin-12*). Significant overexpression of *galectin-8* mRNA was also noticed in 60% ($n=24$) of cancer with lower TNM stages (Stages 1 /2 versus stages 3 /4; $p=0.001$). No other correlation between clinicopathological features of papillary thyroid carcinoma and galectin family mRNA expression was noted.

Galectin-1 and galectin-3 protein expression

Our results show that galectin-1 protein was more strongly expressed than galectin-3 protein in papillary thyroid carcinoma.

Galectin-1 protein expression was studied in 56 papillary thyroid carcinomas, 30 lymph nodes with metastatic papillary thyroid carcinoma and 7 non-cancer thyroid tissues. Normal or under-expressed levels of the galectin-1 protein were found in 68% ($n=38$) of the primary papillary thyroid carcinoma tissue, whilst it was over-expressed in 32% ($n=18$) of the carcinomas (Table 2) (Figure 2). Lymph nodes that had cancer metastases had significantly greater expression levels of galectin-1 protein with 53% ($n=9/17$) of the specimens classified as having increased expression ($p<0.05$). Dichotomising the primary thyroid carcinoma

group into those without lymph node metastasis and those with lymph node metastasis showed that 50% (n=14/28) of the lymph node positive specimens had overexpression of galectin-1 protein compared to the lymph node negative group ($p < 0.05$).

Galectin-3 protein expression was studied in 61 papillary thyroid carcinomas, 30 lymph nodes with metastatic papillary thyroid carcinoma and 7 non-cancer thyroid tissues. Normal or under-expressed levels of the galectin-1 protein were found in 85% (n=52) of the primary papillary thyroid carcinoma tissue, whilst it was over-expressed in 15% (n=9) of the carcinomas (Table 3) (Figure 3).

No significant differences for protein expression of galectin-3 were noted in the primary thyroid cancer or associated lymph nodes. However, expanding the analysis further by splitting the primary thyroid carcinoma group into those with lymph node metastasis and without lymph node metastasis showed that a significant number of thyroid carcinoma with lymph node positive specimens (36%) had a greater expression of galectin-3 compared to those thyroid carcinoma without lymph node metastasis (0%) ($p < 0.05$).

Although increased expression of galectin-1 protein was noted in higher T stage papillary thyroid carcinomas, it did not reach statistical significance. On the other hand, 35% of T3 papillary thyroid carcinomas demonstrated a significantly higher level of galectin-3 protein expression compared to T1 or T2 carcinomas ($p < 0.05$).

Other parameters

In this study, we noted that mRNA expression and protein expression was correlated in galectin-1 and galectin-3 in 64% and 46% of cases respectively. Also, no other associations were found between galectin-1 or 3 protein expressions and other clinicopathological features of papillary thyroid carcinoma.

Discussion

The results presented in this research support those in the current literature for a potential role for galectin family in cancer pathogenesis and metastasis. This study has shown significant mRNA expression differences in galectin family members in primary papillary thyroid carcinomas, especially in metastasis focused subcategories. In this study, there was a significant reduction in expression of *galectin 7* mRNA noticed in papillary thyroid carcinomas with lymph node metastasis compared to non-metastatic primaries. In previous studies, lower expression of galectin-2 and 7 has been associated with gastric cancer and its metastatic behaviour, indicating a potentially similar mode of action [27, 28]. Lower expression of galectin-7 protein has also been previously reported in 17 papillary thyroid carcinomas compared to benign lesions [29]. Additionally, significant mRNA expression differences in papillary thyroid carcinoma, including, importantly, the significant potential role of *galectin-4, 8, 9 and 10* mRNA in metastatic papillary thyroid carcinomas has been demonstrated for the first time in this study. The increased expression of galectin-4 has been proposed as an independent predictor for lymph node metastasis in lung adenocarcinoma [30]. We showed here that the expression of *galectin-4* in our study was lower in papillary thyroid carcinoma of lower T stages, indicating a similar relationship with invasion and metastasis, though metastatic primaries could not be significantly differentiated from non-metastatic primaries. Interestingly, the spread of data for *galectin-4* expression in lymph node metastases in our study indicates that there may be two sub-populations of lymph node metastases, one with high and one with low expression of the gene, a phenomenon which would bear further investigation as to whether these metastases have different gene expression profiles that may lead to different patient outcomes if they remained untreated.

In this study, overexpression of *galectin-8* was noticed in papillary thyroid carcinoma at their primary development stage, followed by further increases in metastatic lymph nodes.

The role of this gene in the pathogenesis of thyroid carcinoma has also been studied in protein level in different thyroid carcinomas, and the overexpression of *galectin-8* in papillary thyroid carcinoma has been suggested as a potential diagnostic tool [31]. The multifunctional roles of *galectin-9* in tumor progression, survival and metastasis have been reported in liver, breast, cervical cancer [32-34]. Galectin-10 or Charcot-Leyden crystal protein has been found mainly in inflammatory reactions and rarely investigated in any malignancies [35]. Thus, the significant increases in expression, trending with increased malignancy and metastasis for both *galectin-9* and *galectin-10* observed in this research warrants further investigation to determine if their role in thyroid cancer matches what has been seen in other cancer types. Finally, a growth regulatory role for galectin-12 has been hypothesized after its introduction to cervical cancer cell line resulted in growth arrest in G1 phase and a halt in growth [36]. In our correlative data, we also noticed a significant link between the expression level of *galectin-12* and the expansion and the size of the tumour, known as T stage, in papillary thyroid carcinoma. Our data also shows a significant reduction in all subtypes of malignant thyroid tissue compared to controls, indicating that high *galectin-12* expression may also hinder the growth of thyroid cancers.

In thyroid cancer, nearly all the previous studies focused on the galectin-3 protein in the diagnosis of papillary thyroid carcinoma [3]. In this study, we noted that many members of the galectin family are involved in the pathogenesis of thyroid carcinoma. Overall, the differences in expression seen in our results these series of galectin genes should be recruited for further investigation of their potential roles in metastatic nature of cancer, and especially in thyroid carcinomas.

In this study, there was a normal to overexpressed protein expression profile for galectin-1 in the primary papillary thyroid carcinoma under analysis. The overexpression of galectin-1 in papillary thyroid carcinoma with lymph node metastases and secondary lymph

node lesions in this research concurs with previous reports of papillary carcinomas having an increased expression of galectin-1 [11]. In the primary tumour microenvironment, it has been suggested that galectin-1 may minimise the immune response to cancer through inhibiting T-cells [1]. This protein may also be responsible in assisting circulating cancer cells to arrest on the extra-cellular matrix at capillary beds and secondary organs to develop into metastases [3, 6]. Indeed, depleting galectin-1 in cancer results in significant cancer growth delay [37, 38]. The increased expression of the galectin-1 protein in lymph nodes with metastatic carcinoma and the primary carcinoma in this study may suggest just this kind of local role in the tumour environment, such as immunosuppression, and an influence on metastasis for galectin-1. This study confirms the potential for galectin-1 as a target to disrupt the progress of papillary thyroid carcinoma.

Previous research has demonstrated that *galectin-1* mRNA levels increase significantly in metastatic thyroid cancer [4]. The results presented here for 65 papillary thyroid carcinoma specimens indicate that many carcinomas had decreased levels of *galectin1* mRNA expression in the primary carcinoma tissue. mRNA levels were only increased in 40% of primary papillary thyroid carcinomas that had metastasised to the lymph nodes. However, when metastatic primary tissues are compared to non-metastatic tissues, there is a significant increase in expression, which does concur with previous results. There was also a further significant increase in metastatic lymph nodes. The amount of *galectin-1* mRNA produced in cancer tissues was significantly less than the levels of protein expression detected in this study would indicate, though correlation between the two was significant. An explanation for this discrepancy may be a change in the way mRNA is produced and turned over in cancer tissues compared to normal thyroid. A loss of controlling micro-RNA or other post-transcriptional mechanisms may mean that tumours are able to produce relatively high levels of galectin-1 protein using less mRNA than normal tissues.

Correlations between mRNA expression (abundance) and protein expression normally accounts for approximately 40% as a result of the complex regulatory process of mRNA translation into protein [39]. In this study, we noted that mRNA expression and protein expression was correlated in galectin-1 and galectin-3 in 64% and 46% respectively. Thus, these discrepancies in our results are within the range expected and likely reflect the normal latency and post-transcriptional regulation of mRNA to protein production.

Expression of the galectin-3 protein is often proposed to be a marker to differentiate follicular variant of papillary thyroid carcinoma from other benign follicular lesions of thyroid [3]. However, galectin-3 protein has been noted to be a non-specific marker for cancer as it can be seen in follicular adenomas and multi-nodular goitres [14]. In this study, we noted that only 4% of papillary thyroid carcinoma overexpressed galectin-3 protein and 33% overexpressed galectin-1 protein as compared to non-cancer tissue. Also, galectin-1 showed a stronger expression than galectin-3 in papillary thyroid carcinoma (Figure 2 and Figure 3). Thus, in pathological practice, galectin-3 may not be as useful as has been proposed as a cancer maker in papillary thyroid carcinoma. In fact, galectin-1 may be a stronger predictor of malignancy than galectin-3.

Prior studies have found the galectin-3 protein [7, 8, 12-14, 16, 17, 20, 40, 41] and galectin-3 mRNA [21, 22] are overexpressed in papillary thyroid carcinoma. In this study, we found that those thyroid carcinomas with lymph node metastases had a greater expression of galectin-3. The literature has generally suggested that the more invasive and aggressive papillary thyroid carcinomas are, the more likely it is that galectin-3 will be overexpressed [3, 42, 43]. Turkoz and colleagues debated these findings, where the authors demonstrated a lower expression of galectin-3 in lymphatic metastases and their primary carcinomas [18]. It has been suggested that the decreased galectin-3 may loosen the connection of cancer cells with the extracellular matrix to facilitate the invasive properties of cancers [7]. The results

presented here do not support this latter theory, rather suggesting an increased level of galectin-3 expression may be facilitative for cancer metastasis where the protein may act to modulate cell growth and be involved in the regulation of apoptosis [5, 42].

Further supporting the role galectin-3 may have in the regulation of growth and apoptosis in thyroid carcinoma are the results presented here that demonstrate a significant correlation between increased levels of galectin-3 expression and a greater tumour size (increased T stage). This has been demonstrated elsewhere in the literature, where papillary thyroid carcinomas with decreased levels of galectin-3 expression have smaller overall tumour size [44].

This study has added to and expanded upon the role previously proposed for galectin-1 by Xu and colleagues who suggested that it might be a marker for thyroid malignancy [11]. Expression of galectin-1 also increased here when comparing thyroid malignancies to non-cancer thyroid tissue. In addition, the most significant result noted in this study was the increase in galectin-1 expression for not only the primary thyroid carcinomas that had metastases, but also for their metastatic carcinoma in the lymph nodes. The overexpression of galectin-1 in cancers that have metastasised suggests that galectin-1 has a role in advancing the papillary thyroid carcinoma from its primary location to a distant locale. Galectin-1 may be expressed by the carcinoma to ensure its ability to bind to the extra-cellular matrix in capillary beds and distal organs that will ultimately enhance the metastatic potential of the carcinoma. Studies investigating other cancer types support this hypothesis. Kim and colleagues demonstrated an association between high galectin-1 expression in primary cervical cancer with an increased rate of lymph node metastasis and deep stromal invasion [37]. The research presented here is a significant addition to the literature that identifies galectin-1 as a potential target for therapies to combat cancer metastasis, as all evidence thus

far indicates that it has a role in transitioning cancer from its local site to widespread invasiveness.

The role for galectin-3 in tumour pathogenesis is less clear. Many series have reported a widespread increase in this protein for thyroid cancer. In contrast to these results, this study has documented minimal differences between galectin-3 protein expression in normal and cancerous thyroid tissue. A significant increase in galectin-3 (mRNA and protein) expression in the primary thyroid carcinomas was found in this research, but only when they had lymph node metastases. In another study, increased serum concentrations of galectin-3 in patients with metastatic disease have also been found, suggesting that the protein could enhance adhesiveness between tumour cells and the extracellular matrix, similar to galectin-1, or promote tumour cell embolization [45]. Galectin-3's exact role in metastasis has been debated despite these results, as it has been found to be dominantly expressed in the cytoplasm, which argues against an active role in cell-to-cell interactions [40]. Instead, galectin-3's role may be in modulating the surface expression of integrins to indirectly influence cellular adhesion properties [46]. Clearly, it is not a simple relationship galectin-3 has with thyroid cancer pathogenesis and metastasis and more research needs to be conducted that focuses away from expression levels to analyse the intra- and extra-cellular role this protein has in the cancerous cell.

Our results show that galectin-1 and galectin-3 protein productions in papillary thyroid carcinoma increase when there has been metastasis to the surrounding lymph nodes. The significance of mRNA expression levels of galectin-1 and galectin-3 were less clear in this study, but this may be due to the degradation [13] and slow production [39] of mRNA compared to the related proteins. We hypothesise that galectin-1 has an important role in facilitating cell-to-cell interactions in cancer during metastasis, and that galectin-3 has a yet undetermined function in also progressing a carcinoma to one with metastatic potential that

may be through modulating the cell cycle or signalling cascades to influence cell-surface protein expression. This research has further supported the current literature in identifying galectin-1 and galectin-3 as possible contributors to facilitating the metastatic potential for papillary thyroid carcinoma, and further efforts should be directed at silencing the production of these proteins to fully elucidate their function.

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Figure Legends

Figure 1

Galectin family mRNA expression in papillary thyroid carcinoma with lymph node metastasis (PTC LN+) and without lymph node metastasis (PTC LN-) compared with secondary metastatic papillary thyroid carcinoma in lymph node (LN) and non-cancer thyroid tissue control (Non-cancer). Y-axis of each graph represents inverse ratio of mean CT value (RR) of each galectin (Glc).

Figure 2

Galectin-1 protein expression in (A) papillary thyroid carcinoma; (B) metastatic carcinoma in lymph node (B) ($\times 20$)

Figure 3

Galectin-3 protein expression in (A) papillary thyroid carcinoma; (B) metastatic carcinoma in lymph node (B) ($\times 20$)

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	AGACAATGGTGGATTTCGCT
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	CTTAGGCTGCCATTTCGCT
		Reverse	AAGCTTTTGGCATTTCGA
Galectin-9	Product 91	Forward	CTTTCATCACCAACCATTCTG
		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

Table 2**The relationship between clinicopathological features and Galectin-1 protein expression in papillary thyroid carcinoma**

Parameters	Galectin-1 protein expression		p-value
	Under expression or Normal	Over expression	
Age (year)			
<45	22(39%)	9(16%)	0.579
≥45	16(29%)	9(16%)	
Gender			
Male	15(27%)	5(9%)	0.394
Female	23(41%)	13(23%)	
T stage			
1 + 2	27(48%)	9(16%)	0.125
3 + 4	11(20%)	9(16%)	
Lymph node metastasis			
Present	14(25%)	13(23%)	0.013
Absent	24(43%)	5(9%)	
Psammoma bodies			
Present	18(32%)	10(18%)	0.567
Absent	20(36%)	8(14%)	
Osseous metaplasia			
Present	1(2%)	1(2%)	0.582
Absent	37(66%)	17(30%)	
Calcification			
Positive	22(39%)	12(21%)	0.530
Negative	16(29%)	6(11%)	
Size (mm)			
<20	13(23%)	7(12%)	0.877
20-40	19(34%)	9(16%)	
>40	6(11%)	2(4%)	

Table 3**The relationship between clinicopathological features and Galectin-3 protein expression in papillary thyroid carcinoma**

Parameters	Galectin-3 protein expression		p-value
	Under expression or Normal	Over expression	
Age (year)			
<45	31(51%)	4(6%)	0.395
≥45	21(35%)	5(8%)	
Gender			
Male	17(28%)	5(8%)	0.187
Female	35(58%)	4(6%)	
T stage			
1 + 2	38(62%)	3(5%)	0.019
3 + 4	14(23%)	6(10%)	
Lymph node metastasis			
Present	18(31%)	9(16%)	0.0004
Absent	31(53%)	0(0%)	
Psammoma bodies			
Present	23(37%)	7(12%)	0.063
Absent	29(48%)	2(3%)	
Osseous metaplasia			
Present	1(2%)	1(2%)	0.153
Absent	51(83%)	8(13%)	
Calcification			
Positive	29(48%)	7(12%)	0.215
Negative	23(37%)	2(3%)	
Size (mm)			
<20	20(34%)	2(3%)	0.529
20-40	26(42%)	5(8%)	
>40	6(10%)	2(3%)