ORIGINAL ARTICLE

Low meprin α expression differentiates primary ovarian mucinous carcinoma from gastrointestinal cancers that commonly metastasise to the ovaries

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Background: Currently, no specific immunohistochemical markers are available to differentiate primary mucinous epithelial ovarian cancer (MOC) from adenocarcinomas originating at other sites that have metastasised to the ovary, which may have an impact on patient management and prognosis.
Aim: To investigate the expression of two intestinal markers, galectin 4 and meprin α, in mucinous carcinomas of the ovary and gastrointestinal tract.
Methods: Using immunohistochemical analysis, the expression of galectin 4 and meprin α was investigated in 10 MOCs and in 38 mucinous adenocarcinomas of colon, pancreas, stomach and appendix, the most common sites of origin of ovarian metastases.
Results: Total cytoplasmic galectin 4 expression was relatively consistent between the different carcinomas. Membranous meprin α expression was significantly lower in MOCs compared with gastrointestinal carcinomas. Moreover, meprin α expression showed greater discrimination between the ovarian and gastrointestinal carcinomas than the cytokeratins CK7 and CK20, the current standard immunohistochemical markers used to determine the tissue origin of mucinous carcinomas involving the ovaries.
Conclusions: Meprin α is a useful additional marker in differentiating primary from secondary mucinous adenocarcinomas of the ovary.

The differentiation of primary mucinous epithelial ovarian carcinoma (MOC) from ovarian metastatic disease arising from other sites, most commonly the gastrointestinal tract, is a major diagnostic challenge.[1–4] Indeed, many carcinomas diagnosed as primary MOC are probably metastatic disease.[3 5] Accurate classification of tumour origin affects patient management, including surgical options and chemotherapy agents, and therefore patient outcome.[6]

Ovarian metastases from gastrointestinal primary tumours can have a similar histological appearance to primary MOC,[4] and therefore immunohistochemical analysis is often required to distinguish between primary and metastatic disease. In particular, differential cytokeratin (CK7 and CK20) expression is widely used to assist in the identification of the probable site of tumour origin. Generally, ovarian primary mucinous tumours are positive for CK7 expression, with variable (focal or diffuse) CK20 staining, and intestinal carcinomas are normally diffusely CK20 positive and CK7 negative.[4 7 8] More recently, CDX2 has been suggested as an indicator of a primary intestinal carcinoma.[9–12] However, there is considerable overlap in the immunohistochemical profiles of primary and metastatic carcinomas with these markers, and there is a clear need for additional targets.

Recently, we performed a gene expression profiling study and found that primary MOCs are distinguished from the other subtypes of epithelial ovarian cancer by the expression of many intestinal-type genes.[13] Several of these genes are expressed in gastrointestinal carcinomas, including galectin 4, a carbohydrate-binding cell adhesion molecule normally expressed in epithelial cells of the alimentary tract, including the intestine. Galectin 4 is overexpressed in a number of cancers, including breast, liver and gastric carcinomas.[14] Both reduced expression[15] and overexpression[14 16] of galectin 4 have been reported in colorectal cancer. Meprin α is a secreted and plasma membrane-bound metalloprotease of intestinal and kidney epithelium, which is often upregulated in cancer, including colon and breast carcinomas.[17 18] Because of its ability to cleave extracellular matrix proteins, meprin α is implicated in tumour invasion and metastasis.[18 19] Our expression-profiling study suggested that MOCs were characterised by high galectin 4 and low meprin α expression.[13] We hypothesised that expression of these markers may therefore be able to discriminate between primary MOC and ovarian metastases from gastrointestinal carcinomas. To this end, using immunohistochemical analysis, we determined expression of galectin 4 and meprin α in primary MOC and gastrointestinal cancers from the most common tumour sites of origin of ovarian metastases, including mucinous colorectal carcinomas, appendiceal adenocarcinomas, gastric mucinous/signet-ring cell carcinomas and pancreatic mucinous (colloid) carcinomas.[4 7] The ability of these markers to aid in the histopathological distinction of primary MOC from secondary ovarian cancers was compared with expression of CK7 and CK20 in this tumour cohort.

MATERIALS AND METHODS

Tissue samples

Tissue specimens (formalin-fixed, paraffin-wax-embedded samples) were collected from patients at the Gynaecological Cancer Centre, Royal Hospital for Women, Randwick, New South Wales, Australia, the Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia, the Royal North Shore Hospital, St Leonards, New South Wales, Australia,

Abbreviation: MOC, mucinous epithelial ovarian cancer

Westmead Hospital, Sydney, Australia, Concord Hospital, Sydney, Australia, and St Vincent's Hospital, Darlinghurst, New South Wales, Australia, after obtaining informed consent and approval by the appropriate hospital research ethics committee (South Eastern and South Western Sydney Area Health Service Research Ethics Committees, St Vincent's Hospital Human Research Ethics Committee, Sydney South West Area Health Service Ethics Review Committee, and Human Research Ethics Committee of the Royal North Shore Hospital, North Shore Private Hospital and the Mater Misericordiae Hospital, Sydney). All specimens were subjected to specialist pathological review (RAS, JPS) before inclusion in the study. All of the MOC samples were diagnosed as FIGO (International Federation of Gynecology and Obstetrics) stage I unilateral primary mucinous adenocarcinomas of the ovary, defined by the absence of clinical, morphological or microscopic features that suggested metastatic disease, including concurrent gastrointestinal tumours, presence of *Pseudomyxoma peritonei/ovarii*, bilateral disease or Krukenberg tumours.[1–4 7]

Immunohistochemistry

Expression of each marker was determined in tissue samples from 10 patients with primary MOC, 14 mucinous colorectal carcinomas, 11 gastric signet-ring cell carcinomas, 11 pancreatic colloid (non-cystic) carcinomas and 2 appendiceal adenocarcinomas. After dewaxing and rehydration, 4 μm tissue sections cut from archival paraffin wax blocks or tissue microarrays were treated with 3% H_2O_2 to block endogenous peroxidase and proteinase K to facilitate antigen retrieval, followed by incubation with primary antibody for 1 h (rabbit anti-meprin α, 1:100 (E Sterchi, Berne, Switzerland); goat anti-galectin 4, 1:100 (sc19286, Santa Cruz Biotechnology, Santa Cruz, California, USA); mouse anti-CK7, 1:100 (M7018, DAKO); mouse anti-CK20, 1:50 (M7019, DAKO)). Bound antibody was detected using LSAB+ Kit/Horseradish peroxidase and DAB+ (diaminobenzidine; DAKO, Glostrup, Denmark), followed by haematoxylin staining. Negative controls omitted the primary antibody. The percentage of cells staining and average intensity of staining were scored by two independent observers (JPS, RAS). In the case of a discrepancy in results between the observers, a consensus result was determined by combined review of each case. Immunohistochemical analysis scores were calculated using the multiplicative Quickscore method as described.[20] Briefly, the percentage of positively staining cells was assigned a nominal score as follows: 0–4%, 1; 5–19%, 2; 20–39%, 3; 40–59%, 4; 60–79%, 5; and 80–100%, 6. The average intensity of staining across the tissue sample was assigned a value from 0 to 3. Values were then multiplied to create a relative score (0–18).

Statistical analysis

The mean staining for each marker and differences in protein expression between groups using the Mann–Whitney U test were calculated using Statview V.4.5 (Abacus Systems, Berkeley, California, USA).

RESULTS

We first examined CK7 and CK20 expression to determine differential cytokeratin expression in our patient cohort. The mean score for CK7 expression in the MOC samples was 11.6/18 (64.4%), with a mean CK20 expression of 9.6 (53.3%; table 1). CK7 expression was significantly lower in mucinous colorectal carcinoma compared with MOC (3.1 (17.2%), $p = 0.007$), but CK20 expression was not significantly different (12.3 (68.3%), $p = 0.50$). The gastric signet-ring cell (16.6 (92.2%)) and pancreatic colloid carcinomas (16.4 (91.1%)) had consistently high CK7 expression, both of which were significantly higher than in MOC ($p = 0.02$ and 0.03, respectively). There was no significant difference in CK20 expression compared with MOC in both carcinomas. The appendiceal carcinomas tested did not express CK7, and had variable CK20 expression (6 (33.3%)), which was not significantly different from CK20 expression in MOC. Although this pattern of CK7/CK20 expression generally agrees with previously reported data for appendiceal carcinomas,[7 21] the sample number is small ($n = 2$), because of difficulty in sourcing appropriate cases.

Figure 1 shows examples of the staining pattern for galectin 4 and meprin α in each of the carcinomas. Galectin 4 staining was diffusely cytoplasmic, similar to that previously observed in colorectal and breast adenocarcinoma.[14] Meprin α expression was both cytoplasmic and on the cell surface, predominantly in an apical location. This pattern is consistent with its known cellular location as being both extracellular (secreted) and plasma membrane-bound when complexed to the meprin β protein.[17]

High galectin 4 expression was evident in each carcinoma type. Although all of the carcinomas (excluding the appendiceal) had a similar overall percentage of cells staining positively for galectin 4 (data not shown), staining in the MOC and mucinous colorectal carcinomas was less intense, resulting in a lower mean score (8.3 (46.1%) and 9.9 (55.0%), respectively) than that for galectin 4 staining in the gastric (13.2 (73.3%)) and pancreatic (14.5 (80.6%)) carcinomas (table 1). These differences were not statistically significant. Galectin 4 expression in the appendiceal carcinomas was lower than in MOC (3 (16.7%)), but this was also not significant.

Membranous (apical) meprin α staining was significantly lower in MOC (1.7 (9.4%)) than in mucinous colorectal carcinoma (8.1 (45.0%), $p = 0.02$) and pancreatic colloid carcinoma (6.3 (35.0%), $p = 0.046$; table 1). Despite a higher mean score (5.3 (29.4%)), 6 of the 11 gastric carcinomas did not

Table 1 Mean Quickscore values (percentage nominal×average intensity = n/18) and percentage of total score (in parentheses) of galectin 4, meprin α, CK7 and CK20 expressions in mucinous ovarian, colorectal, gastric signet-ring cell, pancreatic colloid and appendiceal carcinomas

	Galectin 4	Meprin α	CK7	CK20
Mucinous ovarian (n = 10)	8.3 (46.1)	1.7 (9.4)	11.6 (64.4)	9.6 (53.3)
Mucinous colorectal (n = 14)	9.9 (55.0)	8.1 (45.0)	3.1 (17.2)	12.3 (68.3)
Gastric signet-ring cell (n = 11)	13.2 (73.3)	5.3 (29.4)	16.6 (92.2)	3.0 (16.7)
Mucinous pancreatic (n = 11)	14.5 (80.6)	6.3 (35.0)	16.4 (91.1)	5.4 (30.0)
Appendiceal (n = 2)	3.0 (16.7)	4.5 (25.0)	0 (0)	6.0 (33.3)

CK, cytokeratin.

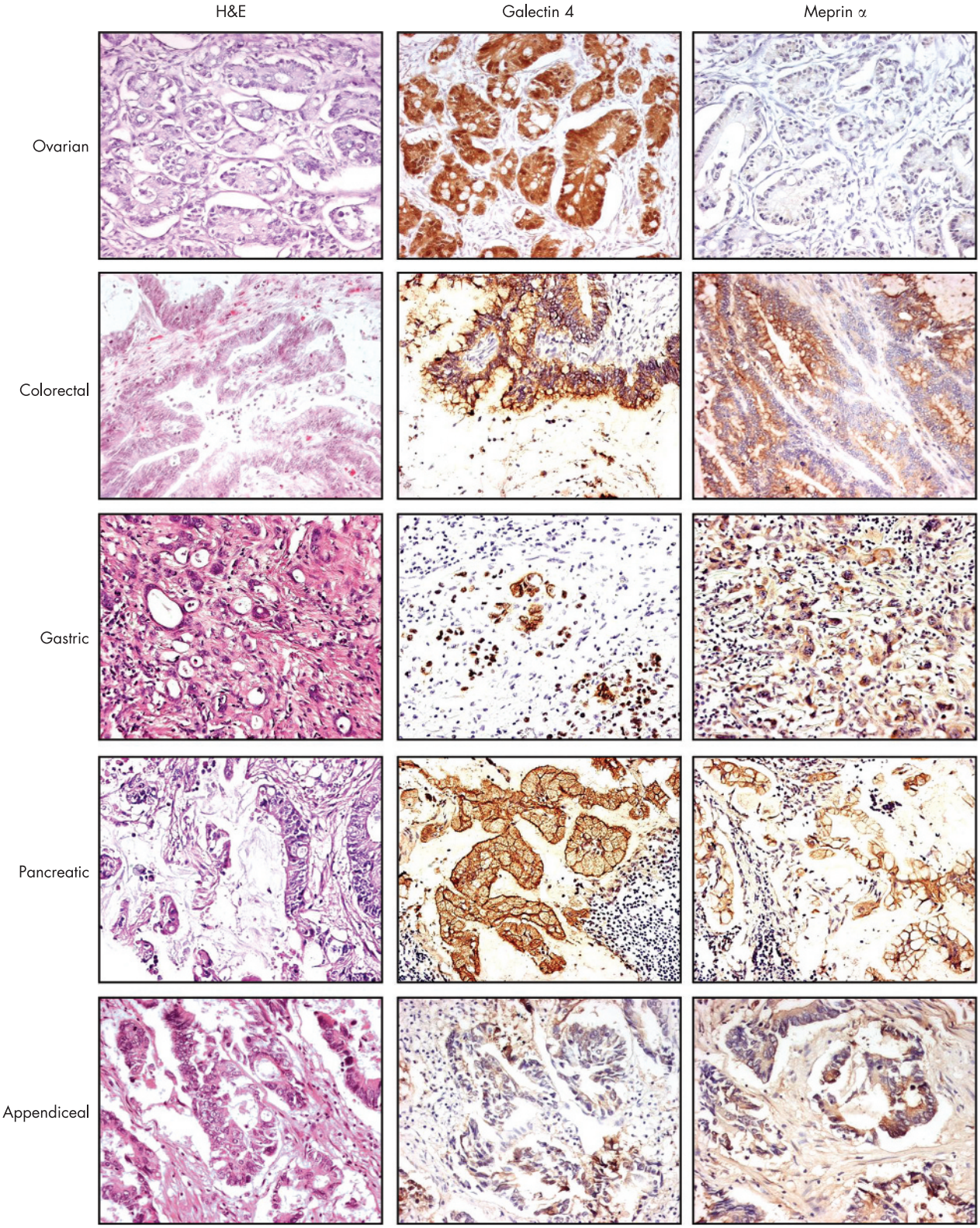

Figure 1 H&E and immunohistochemical expression of galectin 4 and meprin α in mucinous ovarian and gastrointestinal carcinomas. Magnification, ×20.

express meprin α (data not shown), and statistical analysis showed that meprin α expression did not differ significantly from MOC ($p = 0.45$). However, if present, meprin α staining in the gastric carcinomas was of high intensity (overall score 2 or 3) compared with the MOC, which consistently exhibited only low intensity (overall score 1) staining. Moreover, if low-intensity staining was treated as negative staining, the mean percentage of cells with positive membrane expression for

Take-home messages

- Like many gastrointestinal adenocarcinomas, mucinous ovarian carcinomas express high levels of the intestinal adhesion molecule galectin 4.
- Mucinous ovarian carcinomas can be differentiated from mucinous colorectal and pancreatic adenocarcinomas by their expression of low levels of the metalloprotease meprin α.

meprin α in MOC would be only 1.9%, compared with gastric carcinomas at 30%, mucinous colorectal carcinomas at 46.4% and pancreatic carcinomas at 33.2% (data not shown). The mean score for meprin α expression in the appendiceal carcinomas was 4.5 (25.0%), which was not significantly different from that in MOC. Cytoplasmic staining was similar to membranous staining in the majority of the carcinomas (data not shown).

DISCUSSION

Differential cytokeratin staining is widely used to assist in determining the probable site of tumour origin when metastatic carcinoma to the ovary is to be excluded. Although CK7 positivity and CK20 negativity are normally considered to indicate an ovarian primary, this staining pattern is common in adenocarcinomas that commonly metastasise to the ovary, including pancreatic and gastric carcinomas. Our data are consistent with this finding, that the pancreatic colloid and gastric signet-ring cell carcinomas almost exclusively express high levels of CK7 with low or absent CK20. Conversely, high CK20 with absent CK7 is usually indicative of a colorectal carcinoma. However, like other studies, we found that most colorectal carcinomas exhibited at least some focal CK7 staining. Hence, differential cytokeratin staining, although useful, cannot conclusively differentiate between mucinous carcinomas of ovarian and gastrointestinal origin. Other markers have been suggested as useful in determining tissue origin, including CDX2, an intestinal-specific transcription factor. CDX2 is usually, but not always, overexpressed in colorectal cancer and in other intestinal-type carcinomas, including oesophagus, gastric and pancreatic cancers.[9 22 23] CDX2 is also often expressed in MOC,[9–11 24 25] somewhat limiting its use as a marker of tumour origin; however, when combined with CK7 positivity, it may indicate an ovarian primary.[11] Other novel markers including various mucins, β-catenin and others are currently under investigation.[25 26]

We found that expression of galectin 4, another intestinal-specific marker, was also unable to distinguish between the different tumour types. Although the absolute percentage of cells staining with galectin 4 was relatively consistent between the carcinomas, staining was less intense in MOC and in colorectal carcinomas. As previously reported for colorectal and breast carcinomas,[14] expression of galectin 4 was mainly cytoplasmic, with the absence of cell surface expression found in normal intestinal epithelia. Although extracellular galectin 4 mediates cell adhesion, intracellular expression more likely affects cell growth. For example, overexpression of galectin 4 in vitro causes prolonged survival of kidney epithelial cells.[14] Given that galectin 4 is expressed early in carcinogenesis,[13 14] this phenotype may be advantageous during malignant progression.

The metalloprotease meprin α is either secreted or released from the plasma membrane into the underlying stroma, where it is activated by serine proteases causing cleavage of extracellular matrix proteins and breakdown of stromal structure.[17–19 27] We found that levels of membranous (cell surface) meprin α were consistently lower in MOC than in the other carcinomas tested. Furthermore, detection of membranous meprin α expression alone, in particular high-intensity staining, was better able to predict gastrointestinal origin than differential cytokeratin staining. A combination of meprin α and CK7/CK20 staining did not confer additional power in differentiating the carcinoma types, largely because of the high levels of CK7 in the pancreatic and gastric mucinous tumours. We did not determine expression in secondary MOC diagnosed as being of gastrointestinal origin. Given their metastatic phenotype, we would predict that meprin α expression would also be higher in secondary MOC than in primary MOC. However, this remains to be tested.

Although the MOC tissue samples tested were consistently low for meprin α expression, some individual gastrointestinal cancers also showed absence of meprin α expression—in particular, the gastric signet-ring cell carcinomas. Our data suggest that these cancers are more likely to exhibit consistently high CK7 and low CK20 expression, which is more variable in MOC. Regardless, alternative markers will probably be required to distinguish between these types of carcinomas.

The results of this study indicate that meprin α would be a useful addition to a panel of antibodies for the immunohistological differentiation of primary MOC from mucinous metastatic disease. We suggest that meprin α expression should be examined in an independent cohort of MOC and mucinous gastrointestinal cancers, including those that have metastasised to the ovary, to further assess its application in such a panel.

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