

Expression of Podoplanin in Primary and Metastatic Poorly Differentiated and Undifferentiated Carcinomas of the Head and Neck

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Abstract. *The invasive growth properties of head and neck carcinoma can be determined by proteins associated with cellular motility. Podoplanin is a recently identified protein associated with motility of cells. Materials and Methods: Sixty-five tumour specimens of 51 patients with poorly and undifferentiated carcinomas were investigated for podoplanin expression. All tissues were fixed in buffered formalin and embedded in paraffin. Results: Podoplanin expression showed a tendency towards a more intense staining in carcinomas with a squamous epithelia differentiation compared to tumours without any remnants of cellular layers. Podoplanin expression was very rarely seen in lymph node metastases and the staining was weak in these specimens. The differences of podoplanin expression between primary and metastatic carcinoma were highly significant ($p < 0.005$). Conclusion: Podoplanin is expressed in primary undifferentiated carcinoma, both inside and outside of Waldeyer's ring. This study shows that podoplanin can be used as a marker of tumour invasion in poorly or undifferentiated head and neck carcinoma.*

Tumour biology of poorly and undifferentiated carcinoma of the head and neck is difficult to assess. Some head and neck carcinomas are associated with oncogenic virus, e.g. Epstein-Barr virus (1). Determination of oncogenic viruses appears to be a prognostic marker in some types of head and neck cancer [squamous cell carcinoma (SCC)] and is used as a stratification marker (1). On the other hand, primarily well-

differentiated SCC may become de-differentiated during the course of the disease, in particular in cases with recurrent cancer and previous radiotherapy (2). One important tool for studying the invasive growth properties of head and neck SCC is the determination of proteins related to degradation of tissues and propagation of invasion (3). Regional growth into an adjacent lymphatic vessel is the most important route of spread for head and neck SCC (3).

Podoplanin is a protein expressed in lymphatic vessels (4, 5). In addition, podoplanin has been found in squamous cell carcinoma (6) and in other tissues (7). Recently, the expression of podoplanin was described in head and neck cancer (8, 9). The protein is secreted by carcinoma cells (6) and proved to be a prognostic marker for oral and oropharyngeal SCC (11-13). Furthermore, podoplanin can be used to identify lymphatic vessels in oral and oropharyngeal carcinoma (14). In the present study, the diagnostic significance of podoplanin expression in primary and metastatic carcinomas of the head and neck with poor differentiation was evaluated.

Materials and Methods

Sixty-five tumour specimens of 51 patients were investigated for podoplanin expression (antibody: D2-40, 1:40, Signet, Dedham/MA., USA, 1:40). All tissues were fixed in buffered formalin and embedded in paraffin. Four micrometres thin sections were processed for histological investigation as described elsewhere (15). Normal lymphatic vessels served as internal positive control in all negative cases. Age, gender, type of tumour, evidence of metastasis and immunoreaction of tumour and normal tissues were statistically calculated using non-parametric correlations (Kendall-Tau-b). In six cases the immunoreaction was compared to Epstein-Barr virus presence in carcinoma cells (16).

Results

Podoplanin expression was predominantly identified in tumour cells of primary tumours. Podoplanin expression was

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Key Words: Undifferentiated carcinoma, head and neck cancer, nasopharyngeal carcinoma, podoplanin, Epstein-Barr virus.

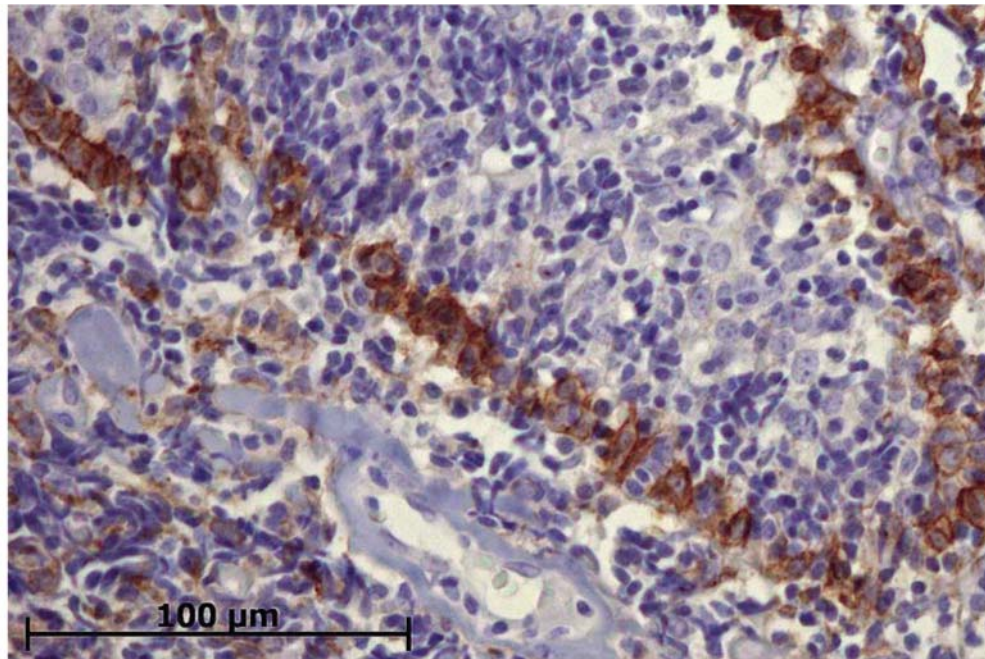


Figure 1. Undifferentiated carcinoma, podoplanin staining of the invasion front (chromogen DAB).

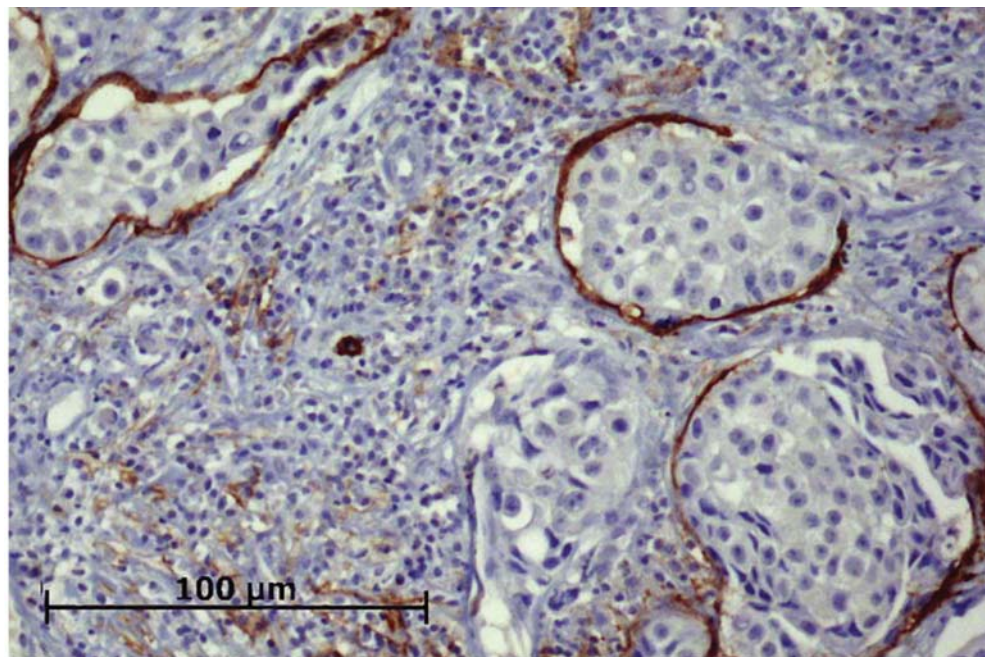


Figure 2. Podoplanin staining of lymphatic vessels in a lymph node of the neck disclosing intraluminal growth of an undifferentiated carcinoma (chromogen DAB).

more intense in carcinomas with a squamous epithelia differentiation than in tumours without any remnants of cellular layers. However, this difference was statistically not significant. Podoplanin expression was very rarely seen in

lymph node metastases and the staining was weak in these specimens. The differences in podoplanin expression between primary and metastatic carcinoma were highly significant ($p < 0.005$). Podoplanin expression was also

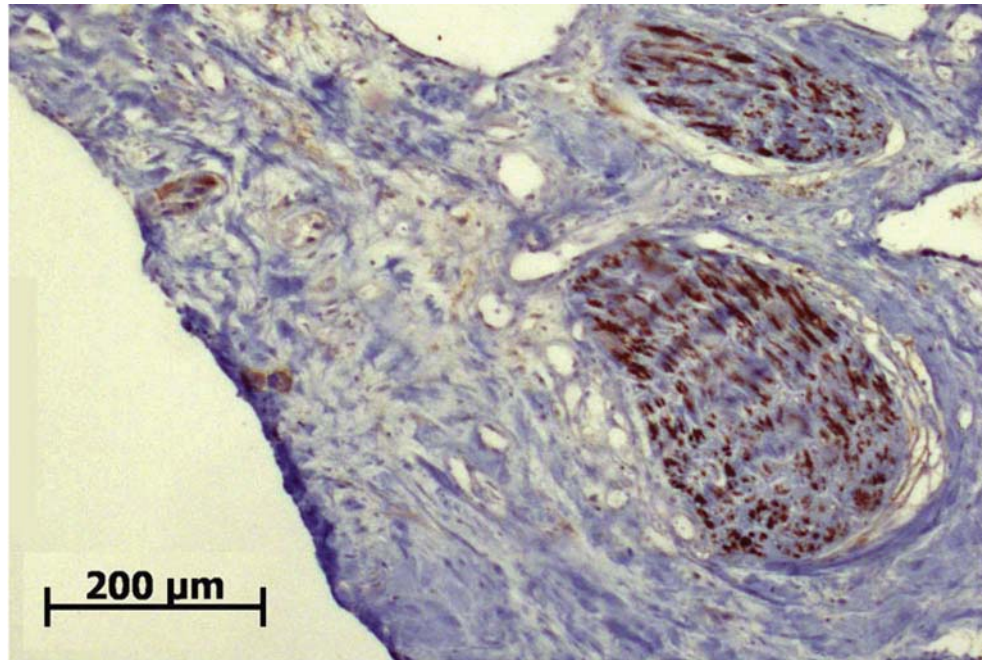


Figure 3. Expression of podoplanin in Schwann cells of nerve fascicles in the vicinity of the tumour (chromogen DAB).

Table I. Association of podoplanin expression and presence of Epstein-Barr virus (EBV).

| No. | Diagnosis | Site | EBV-ISH | Podoplanin in tumour cells | Podoplanin in adjacent normal tissues |
|----------|---|-------------|----------|----------------------------|---------------------------------------|
| 3713.85 | Undifferentiated carcinoma (Schmincke) | Nasopharynx | Positive | Negative | Positive |
| 8518.85 | Undifferentiated carcinoma (Schmincke) | Nasopharynx | Positive | Positive | Negative |
| 12487.86 | Undifferentiated carcinoma (Schmincke) | Nasopharynx | Positive | Positive | Negative |
| 847.87 | Squamous cell carcinoma | Oropharynx | Negative | Positive | Negative |
| 4139.87 | Squamous cell carcinoma | Oropharynx | Negative | Negative | Negative |
| 2342.87 | Undifferentiated carcinoma (Schmincke type) | Lymph node | Positive | Positive | Negative |

ISH=in situ hybridization (16).

altered in adjacent stroma, *e.g.* muscle cells or Schwann cells were occasionally immunoreactive (Figure 3). Podoplanin was a good marker for identifying lymphatic invasion of carcinomas.

Discussion

Podoplanin is expressed in primary undifferentiated carcinoma, both inside and outside of Waldeyer's ring. This study shows that podoplanin can be used as a marker of tumour invasion in poorly or undifferentiated head and neck carcinoma. Previously, expression of podoplanin was shown to be associated with the differentiation of SCC of the skin (6). The protein was not expressed in well-differentiated SCCs, but identified in the basal tumour cell layers of

moderately differentiated SCC. In less-differentiated SCC podoplanin expression was also demonstrated beyond the basal layers and impressed even as cytoplasmic staining (6). All recurrent SCC expressed podoplanin (6). In addition, high levels of podoplanin expression were associated with carcinoma spread to regional lymph nodes (12, 13) and indicative of poor survival (10, 13).

The association of podoplanin expression and tumour differentiation is confirmed in this study on carcinomas derived from mucous epithelia of the upper aerodigestive tract.

Concerning the specificity of podoplanin staining, this protein is not exclusively expressed in carcinoma cells (15). Indeed, the distinction between podoplanin-positive lymphatics and SCC may be difficult to determine (17), in

particular in tissues with abundant myoepithelial cells and prominent basal epithelial cell layers (17). Furthermore, weak immunostaining of blood vessels was noticed in an earlier study on immunophenotyping of lymphatic vessels using a non-commercial antibody to podoplanin (18). Since the carcinoma cells and lymphatic tissues are intertwined in the naso-oropharynx, morphological distinction between lymphatic and epithelial cells may be difficult (19). Therefore, the interpretation of immunoreactions of podoplanin in undifferentiated carcinomas of the head and neck should be performed with caution. On the other hand, the distinction between carcinoma cells immunoreactive for podoplanin and podoplanin-positive adjacent basal epithelia of unaffected tissues was distinct in all cases.

A subset of the tumours was investigated for Epstein-Barr DNA in carcinoma cells (16). Three out of four Epstein-Barr virus-positive undifferentiated carcinomas showed intense podoplanin expression. However, these preliminary results do not demonstrate a significant difference in podoplanin expression in undifferentiated carcinoma bearing Epstein-Barr virus and poorly differentiated SCC of the head and neck.

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