**Abstract.** Background/Aim: Tumour budding (TB) is a specific pathological feature that has been found to be associated with an aggressive outcome in several cancer types; however, to our knowledge, TB has not yet been assessed in squamous-cell carcinomas of the skin (SCC). The aim of the study was to study whether TB correlates with aggressiveness in cutaneous SCC. Materials and Methods: We examined 31 aggressive SCC (that later developed local recurrences or metastases) in comparison with 21 non-aggressive SCC (not complicated by recurrence or metastasis). TB was expressed as the mean number of tumour buds in five adjacent high-power fields of each SCC. Results: Aggressive SCC had a much higher TB score compared to control SCC (1.63±1.35 vs. 0.49±0.9, p<0.001). Conclusion: As with other cancer types, TB seems to be a pathological marker of aggressiveness of cutaneous SCC, along with other features known to be associated with an aggressive outcome (tumour thickness, level of invasion and lymphovascular or perineural invasion). Further studies including a larger number of tumours will hopefully validate TB as a new pathological predictor of aggressiveness in cutaneous SCC and will allow its correlation with other pathological features of SCC aggressiveness to be defined.

Tumour budding (TB) is defined as the process during which single cells, or small groups of neoplastic cells, detach from the main tumour mass in the invasive front and infiltrate the surrounding connective tissue. This feature is associated with an epithelial–mesenchymal transition, i.e. acquisition of a mesenchymal phenotype by neoplastic cells, enabling them to infiltrate the connective tissue surrounding the neoplasm, penetrate into vascular spaces, and thereby reach distant tissues where they settle down and create a distant metastasis (1-4). The process of TB has been recognized as a feature of adverse tumour outcome, as it has been found to correlate with lymphovascular invasion, lymph node and distant metastasis, and decreased patient survival in several solid cancer types, including those of the colon (2-6), oesophagus (5, 8), pancreatic duct (9, 10), lung (11), gallbladder (12), head and neck (13), tongue (14-16), oral cavity (17-18), external auditory canal (19), breast (20, 21) endometrium (22) and stomach (23). TB has been extensively studied in these carcinoma types, and inclusion of this pathological feature in pathological reports has been suggested (2, 3).

Squamous-cell carcinomas (SCC) of the skin are the second most common type of skin malignancy in the population at large, next only to basal-cell carcinomas (24-28), and the commonest malignancy in organ-transplant recipients (OTR), in whom they entail significant morbidity and non-negligible mortality (29-31). Although cutaneous SCCs can usually be cured with surgical excision with adequate margins, some of them have an aggressive course, causing local recurrences and metastases (regional or distant). These events occur in 3-8% and 1.9-10% of cases, respectively (30-32), and result in a mortality rate of 2-5% (32, 33). Several pathological features are recognized as being associated with an aggressive outcome of SCC, i.e. the development of recurrence and metastases and disease-specific death; they include macroscopic (horizontal) size >2 cm, microscopic thickness >2 mm, Clark level of invasion ≥IV, involvement of deep tissues beyond the subcutaneous fat, poor differentiation and intravascular and perineural invasion (34-41). However, the prognostic significance of TB has not yet been studied in SCC of the skin.

The aim of this study was to assess a possible association of TB with SCC aggressiveness. In order to do so, we compared TB in two groups of SCCs (aggressive and non-aggressive) that were excised from a group of OTR followed up by our Dermatology Department.
Materials and Methods

Study design. A retrospective pathological study was performed on biopsy or excision specimens of 31 aggressive SCC that had been excised from a group of OTR followed-up at our specialized outpatient dermatology clinic and that proved upon further follow-up to have an aggressive behaviour, defined as the development of local recurrences or regional or distant metastases, despite an initial surgical excision with tumour-free margins. They included 17 SCCs with subsequent local recurrence and 14 with subsequent metastases (10 to the lymph nodes, six in-transit and four distant, some SCCs developed metastases at more than one site). The skin SCCs were located on the head and neck in 61%, the limbs in 33% and the trunk in 6%. For those that had undergone multiple excisions (because of recurrences), only the initial tumour was examined. Control SCC consisted of 21 SCCs excised from the same group of patients during the 6 months preceding or following excision of the aggressive SCC, matched (whenever possible) for the anatomical location; these non-aggressive SCC had not develop recurrence or metastases during the same follow-up period as that of the aggressive SCC.

Assessment of TB. Representative haematoxylin-eosin-stained sections of each SCC were re-examined by one of us (GK) in a blind fashion as to the outcome of the SCC (aggressive or not) for evaluating the degree of TB. The sections were first examined at low magnification to assess the most invasive areas of each tumour. Thereafter the slides were examined at high magnification (×250) and the number of tumour buds (single cells or groups of up to five malignant cells) in five adjacent microscopic fields was counted. The mean number of tumour buds per high-power field of each SCC, and then per group of SCC (aggressive and non-aggressive) was calculated. Statistical comparison between the two groups was carried out with Student’s t-test, with a p-value of 0.05 or less considered statistically significant.

Results

Tumour buds included single undifferentiated SCC cells or small buds consisting of fewer than five tumour cells. They were found in the dermis in close proximity to the invasive tumour front (Figure 1). The mean score of tumour budding of aggressive SCC and non-aggressive SCC was 1.63±1.35 and 0.49±0.9, respectively. The difference proved statistically significant (p<0.001 with Student’s t-test).

Discussion

TB refers to the presence of clusters of undifferentiated malignant cells in the tumour stroma, located ahead of the invasive front of a tumour (1). TB is nowadays regarded as a pathological feature predictive of poor outcome in several types of cancer, including those of the colon, oesophagus, pancreatic duct, lung, gallbladder, head and neck, tongue, oral SCC, external auditory canal, breast, endometrium and stomach [reviewed in (1)]. Indeed, despite the lack of a universally accepted method of counting TB, studies performed with various methods have almost invariably shown that the degree of TB correlates positively with lymphovascular invasion, recurrence, lymph node metastasis, distant metastasis and poorer patient disease-free and overall survival in several cancer types. TB has mostly been studied in colorectal cancer and is considered by the Union for International Cancer Control as an additional prognostic factor (42), correlated with loco-regional and distant (mostly liver) recurrence and lower 5-year survival. TB also correlates in colorectal cancer with other factors linked to poor prognosis, such as overall stage, T-stage, N-stage, Dukes’ stage, tumour dedifferentiation, infiltrative growth pattern, lymphovascular and perineural invasion, nodal and distant metastasis (1).

Several methods have been applied to study TB. The initial definition of tumour buds included groups of up to four tumour cells (43), however, the actual trend is to consider groups of up to five cells (3), and this value was used in our study. We chose to use a quantitative method for TB counting (6) because this has proven to be the most effective in colorectal cancer (44) and has shown good inter-observer reproducibility (45). Furthermore, recent studies suggest that TB should better be assessed as a continuous variable, rather than considering classes defined by cut-off values (3, 45). In our study, TB was readily performed on routinely stained tissue sections and it was not necessary to use immunohistochemical staining with antibodies against keratins, which can improve the detection of neoplastic cells (46). Using this methodology, we found that aggressive SCC had a significantly higher TB score compared with their non-aggressive counterparts, suggesting that TB correlates with aggressiveness (development of recurrence and metastases) of cutaneous SCC. This finding is not really unexpected, since the degree of TB has been shown to be predictive of an adverse outcome of SCC of other organs, such as the lungs (11), external auditory canal (19) and oral cavity and tongue (13, 14, 16-18).

Several pathological variables have been shown to have a prognostic significance in cutaneous SCC, including microscopic tumour thickness, degree of differentiation, and lymphovascular, perineural and deep-tissue invasion (41). In a previous study, we examined these features in the same groups of tumours as those used in the present study, and found that although the two SCC groups (aggressive and non-aggressive) differed with respect to most of these pathological features, the only statistically significant difference was the invasion level (Clark) (47). This is probably due to the low power of the study because of the limited number of SCC studied. The findings of our present study suggest that TB could be a more sensitive predictor of adverse outcome in cutaneous SCC than the usual pathological features of aggressiveness used to date. The more precise relation of TB with these pathological features is currently under investigation.
The possibility of depicting early (i.e. at the time of initial excision) SCC which are at higher risk for developing local recurrence and metastases is important for adequate patient management, especially in immunocompromised patients, in whom cutaneous SCC may be more aggressive. The results of our study suggest that similar to other cancer types, TB is a significant predictor of the development of recurrence and (locoregional or distant) metastases in cutaneous SCC. Our results should be confirmed by additional studies including a higher number of SCC from the general population and by further assessing whether TB correlates more with recurrence, metastasis, or disease-specific death in SCC. The inclusion of the degree of TB in pathological reports of cutaneous SCC will facilitate such retrospective studies.

Conflicts of Interest

The Authors declare that they have no conflict of interest.

Funding

None.

References


45 Horcic M, Koelzer VH, Karamitopoulou E, Terracciano L, Puppa G, Zlobec I and Lugli A: Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol 44: 697-705, 2013.


Received June 30, 2016
Revised July 14, 2016
Accepted July 15, 2016