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When Is Immunohistochemistry Useful in Assessing Tumor Necrotic Tissue?

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Abstract. *Background/Aim: Immunohistochemistry (IHC) enables visualisation of the distribution of specific proteins, the differentiation of benign and malignant tumours, and the site and origin of a primary tumour. Surgical pathologists commonly examine tumours with extensive necrosis or non-viable tissue that may affect an accurate diagnosis. Materials and Methods: We investigated the sensitivity and specificity of IHC on necrotic samples derived from adenocarcinoma, squamous cell carcinoma (SCC) and melanoma using different markers. Results: Analysis of necrosis within tumours revealed 88% sensitivity and 56% specificity for melanoma, 95% and 92% for CK5/6, 95% and 83% for CK20, 37% and 95% for p63, 69% and 97% for Melan A, 88% and 92% for SOX-10, 98% and 56% for CKAE/AE3 and 75% specificity for CK7. Conclusion: Antibodies should be considered reliable markers for demonstrating the epithelial nature of a suspected tumour. Immunohistochemistry of necrotic tissues may provide clinically useful information.*

Immunohistochemistry (IHC) enables the visualisation of the distribution and levels of specific proteins present in tissues using antigen-specific antibodies. This technique recognises the presence or absence of target proteins in the context of the tissue microenvironment (1). Recent advancements in cell and molecular biology have allowed the development of various antibodies against signal transduction molecules. These antibodies are readily available commercially and are widely used worldwide. IHC is a method that contributes to the diagnosis of neoplasms (2, 3).

IHC can be applied to diagnose cancers because tumour cells show increased expression of specific tumour antigens which can be targeted by known antibodies. IHC enables the differentiation of benign and malignant tumours, as well as, the determination of the site and origin of a primary tumour (4). Therefore, due to these excellent properties, IHC has become a crucial technique for cancer diagnosis and is widely used in clinical and research laboratories (5, 6).

Clinical pathologists and surgeons often find it challenging to diagnose and determine the origin of a tumour, especially when the tumour mass is necrotic, decalcified or autolysed. This problem can be tackled by applying IHC using a panel of known antibodies as well as targeting intermediate filaments such as vimentin, desmin and keratin, to aid in the accurate diagnosis of the type of cancer (7).

Tumour necrosis occurs due to several reasons, namely toxins, trauma, infection or ischemia (8). Evaluation of malignancy is extremely difficult and sometimes impossible in an extensively necrotic specimen. In addition, the resected specimen can undergo ischaemia, which would result in the degradation of proteins and activation of autolysis (9). These processes affect tissue structure and can obscure the underlying pathology, and it is extremely challenging to make a definitive pathological diagnosis (10). It is widely believed that immunophenotypic studies are not suitable for necrotic tissues due to non-specific staining and antigenicity loss (11); however, some studies have challenged this notion (12). For instance, IHC on infarcted lymph nodes in patients with a prior diagnosis of lymphoma has shown preservation of antigens associated with lymphoma (13), as well as with melanoma and metastatic carcinoma (14).

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Table I. *The expression of investigated markers in necrotic areas within tumours.*

	Melanoma TC	CK5/6	CK20	p63	Melan A	SOX-10	CK7	CKAE1/AE3
CRC (cases)	8/20	0/20	19/20	0/20	0/20	3/20	1/20	20/20
SCC (cases)	9/19	18/19	3/19	7/19	1/19	0/19	6/19	18/19
Melanoma (cases)	14/17	3/17	3/17	2/17	11/17	14/17	7/17	7/17
Sensitivity (%)	88	95	95	37	69	88	-	98
Specificity (%)	56	92	83	95	97	92	75	56

CRC: Colorectal adenocarcinoma; SCC: squamous cell carcinoma.

The use of IHC has also shown to play an important role in identifying the primary cause of myelonecrosis. Myelonecrosis is defined as necrosis of medullary stroma and myeloid tissue, which is often associated with malignancy. In myelonecrosis, bone marrow aspiration does not provide adequate cellularity; therefore, IHC plays a major role because it can specify the lineage of these ghost cells (15).

It is a serious challenge for the surgical pathologist to provide accurate diagnosis by examining tumours with extensive necrosis and no viable tissue. Sometimes this leads to cases with no firm diagnosis; therefore, establishing IHC in these necrotic tissues for accurate diagnosis is paramount. Herein, we investigated the sensitivity and specificity of IHC staining for different markers on necrotic tissues. We mainly focused on melanoma, colorectal adenocarcinoma (CRC) and squamous cell carcinoma (SCC).

Materials and Methods

The study was performed on malignant tumours with extensive necrosis, which included 17 cases of melanoma, 19 cases of SCC and 20 cases of CRC. All collected tissue sections were processed according to the standard diagnostic protocol in the Department of Tumour Pathology. Briefly, collected tissue sections were fixed in 10% buffered formalin for 24 hours at room temperature, dehydrated in ethyl alcohols (80%-99.8%), cleared in xylenes (I-IV), and embedded in paraffin. After preliminary evaluation of tissue sections according to hematoxylin and eosin staining, performed by two independent pathologists, tumour sections were selected for immunohistochemical studies. For the immunohistochemical staining, a previously described protocol was used (16, 17). We chose standard, diagnostic markers for melanoma (Melanoma TC, Melan A, SOX-10), for SCC (CK5/6, p63), and for adenocarcinoma (CK20, CK7) as well as one broad marker for cells of epithelial origin such as CK AE1/AE3.

The immunohistochemical studies were performed using the following antibodies: Melanoma TC (06527787001, Roche, Tucson, AZ, USA), mouse monoclonal anti-Melan A (IR633, DAKO, Glostrup, Denmark), rabbit monoclonal anti-SOX-10 (07560389001, Roche), mouse monoclonal anti-CK5/6 (GA780, DAKO), mouse monoclonal anti-p63 (GA662, DAKO), mouse monoclonal anti-CK20 (GA777, DAKO), mouse monoclonal anti-CK7 (GA619, DAKO) and mouse monoclonal anti-CKAE1/AE3 (IR053, DAKO). The brown reaction product indicated the site of the presence of antigen.

The pathologists who were evaluating the immunohistochemical expression of the examined antigens worked independently, and they were blinded regarding the patients' clinical as well as other data. Protein expression was evaluated using light microscope at 20× original objective magnification. In this project, we evaluated immunohistochemical protein expression in malignant tumours and necrotic areas within the tumours. The evaluation score was set as 1 (positive expression) or 0 (negative expression).

Results

Evaluation was performed on living tumour tissue and necrotic masses. The evaluation was scored for both areas separately. The morphological analysis of living tumour cells revealed the expression of melanoma TC in 1/20 cases of CRCs and 17/17 cases of melanoma; CK5/6 in 1/17 cases of melanoma and 18/19 cases of SCC; CK20 in 19/20 cases of CRC and 1/17 cases of melanoma; p63 in 1/20 cases of CRC, 1/17 of melanoma and 19/19 cases of SCC; Melan A in 16/17 cases of melanoma; SOX-10 in all cases of melanoma; CK7 in 1/20 case of CRC and 7/19 cases of SCC; CKAE/AE3 in 20/20 cases of CRC and 18/19 cases of SCC. The morphological analysis of necrosis within tumours revealed expression of Melanoma TC in 8/20 cases of CRC, 9/19 cases of SCC and 14/17 cases of melanoma (sensitivity 88%, specificity 56%); CK5/6 in 3/17 cases of melanoma and 18/19 cases of SCC (sensitivity 95%, specificity 92%); CK20 in 19/20 cases of CRC, 3/19 cases of SCC and 3/17 cases of melanoma (sensitivity 95%, specificity 83%); p63 in 7/19 cases of SCC and 2/17 cases of Melanoma (sensitivity 37%, specificity 95%); Melan A in 11/17 cases of melanoma (sensitivity 69%, specificity 97%); SOX-10 in 3/20 cases of CRC and 14/17 cases of melanoma (sensitivity 88%, specificity 92%); CK7 in 1/20 cases of CRC, 6/19 cases of SCC and 7/17 cases of melanoma (specificity 75%); CKAE/AE3 in 20/20 cases of CRC, 18/19 cases of SCC and 7/17 cases of melanoma (sensitivity 98%, specificity 56%). All results are summarized in Table I and Figure 1.

Discussion

It is often a challenging task for a pathologist to provide diagnosis from an extensively or completely necrotic tumour mass. Necrosis may be encountered in surgical resection specimens of the suspected tumour, metastatic cancer or in a biopsy from a necrotic part of a tumour (14). Necrosis can

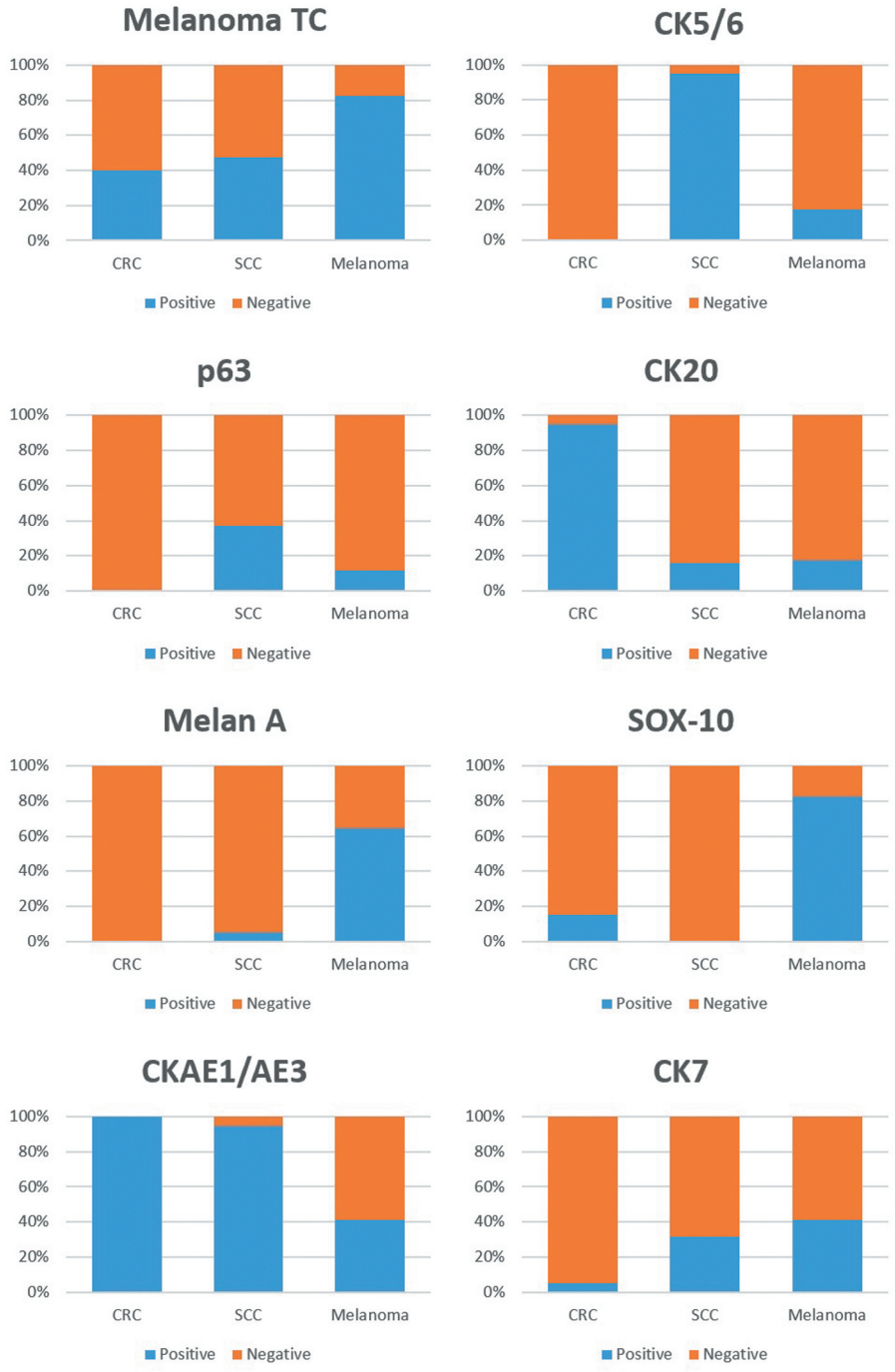

Figure 1. *Graphical presentation of investigated markers expression in necrotic areas within tumours. CRC: Colorectal adenocarcinoma; SCC: squamous cell carcinoma.*

occur; 1) in rapidly developing tumours undergoing necrosis/apoptosis; 2) in neoplasms with a known inclination for spontaneous infarct or induced by fine needle aspiration biopsy (FNA); 3) by FNA performed as an initial diagnostic procedure; and 4) following adjuvant therapy prior to removing the tumour mass (15). IHC can be used to determine the origin of these necrotic tissues by applying a panel of antibodies and further aid with the treatment (18).

Only a limited number of studies have been published regarding the reactivity of antibodies on the necrotic tumour

mass. Most of these studies are either on lymph node infarction or melanoma, but there are very few or no studies describing necrotic adenocarcinoma and SCC. Our study was undertaken to establish the reactivity of necrotic mass derived from three tumour types (CRC, melanoma and SCC) against a panel of known antibodies and observe the preservation of antigens in these necrotic tissues.

CK AE1/AE3 is a common marker to confirm or rule out the epithelial nature of a tissue. All SCCs and adenocarcinomas should be CK AE1/AE3 positive; while, melanoma should be negative. Judkins *et al*. (19) have found that cytokeratin markers such as CK AE1/AE3 have high sensitivity in necrotic thyroid cancers. In agreement, our study showed that CK AE1/AE3 displays very high sensitivity (100%) in CRC and SSC (95%). These findings are similar to those of Nasuti *et al*. who described two cases of SCC with positive immunostaining for CK AE1/AE3, despite extensive necrosis (14). However, in our study, CK AE1/AE3 was not specific (56%) in diagnosing SCC and CRC; low specificity was due to the false-positive results in 60% melanomas. Therefore, additional markers are required for a definitive diagnosis of SCC and CRC with high specificity. Combination of CK5/6 and p63 is commonly used in poorly differentiated non-necrotic carcinomas to detect squamous cell origin with 96% specificity and 77% sensitivity (20). Our study further showed that only CK5/6 could be used as an excellent diagnostic marker on necrotic SCC. This marker gave a high sensitivity (95%) and specificity (92%). Therefore, it can be concluded that CKAE1/AE3 by itself is non-specific, but the combined evaluation of CK5/6 can dramatically increase the sensitivity and specificity in the diagnosis of SCC. P63 was almost negative in all studied cases.

Nonaka *et al*. showed weak expression of Melan A, HMB-45 and tyrosinase in 35 extensively necrotic malignant melanomas (21, 22). In this study, necrotic areas of melanoma were positively stained with varying specificity and sensitivity by all eight markers. Fourteen out of 17 cases of melanoma, 8 out of 20 cases of CRC and 9/19 cases of SCC were positive for Melan-TC, which showed sensitivity 88% and low specificity of 56%. In contrast, Melan-A and SOX-10 were highly specific for melanoma with sensitivity and specificity values of 97% and 92%, respectively. Our study showed that these two markers can be used to diagnose melanoma in necrotic tissues with high sensitivity and specificity. Therefore, the sensitivity/specificity of diagnosing melanoma in necrotic tissue can be increased by combining a panel of Melan-A, Sox-10 and Melan TC.

There is currently no literature on the use of IHC in necrotic tissue originated from CRC. The presence or origin of CRC can be detected by CK20 positive and CK7 negative staining of non-necrotic tissues. In our study 19 of 20 cases of adenocarcinoma were positive for CK20 (95% sensitivity); however, 3 SCC and 3 melanoma cases were also positively stained for CK20, giving a specificity of 83%. It is worth emphasizing that 95% of CRC were negative for CK7. CK20 has been shown to be less sensitive for poorly differentiated colonic carcinoma (23). While CK20 showed promising sensitivity for adenocarcinoma, the low specificity makes this marker less useful. Therefore, other markers such as CDX2 or SATB2 are needed to diagnose CRC from necrotic tissue with high sensitivity and specificity.

Our results demonstrated good preservation of cytoplasmic markers in necrotic tissue. The antibodies against CK5/6, CK20 and CKAE1/AE3 should be considered reliable in showing the epithelial nature of suspected tumour mass especially, SCC. A combined panel of Melan-A and SOX 10 also displayed excellent performance in necrotic melanoma; however, when used individually these antibodies may give false negative results. Immunohistochemistry of infarcted tissues cannot exclusively be used to establish a definitive diagnosis; however, it is a useful tool for further clinical evaluation and may provide clinically useful information when conventional histology fails to give a diagnosis.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

Conception and design of the study: EBR, NA, LS. Methodology and investigation: EBR, BD, VF. Data analysis and interpretation: EBR, AM, LS. Study supervision: AM, LS. Writing – original draft and figure: NA, LS. All Authors have read and approved the final version of the manuscript.

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