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Case Report

Utility of Immunohistochemical Studies in Differentiating Diagnostically Challenging Radiation-Induced Atypia from Recurrent Squamous Cell Carcinoma: A Case Report and Literature Review, with a Proposed Algorithm for Differentiation

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Abstract

Radiation-induced atypia presents a diagnostic challenge in differentiation with squamous cell carcinomas of the upper aerodigestive tract and lung. It is imperative to understand the different features of radiation-induced atypia and differentiate it from squamous cell carcinoma. Immunohistochemistry (IHC) studies play an extremely important role in present-day pathology practice. It is being used for diagnosis of primary and metastatic cancers, as a prognostic marker, targeted therapy, and identification of certain infectious agents. This case report discusses the features specific to each and proposes an IHC algorithm to assist in providing appropriate diagnosis and subsequent optimal management.

Keywords: Radiation; Atypia; Squamous; Carcinoma; Immunohistochemical; Staining; Recurrent; Metastasis; Radiotherapy

Abbreviations

SCC: Squamous Cell Carcinoma; IHC: Immunohistochemistry; NPV: Negative Predictive Value; PPV: Positive Predictive Value; FNA: Fine Needle Aspiration

Introduction

Squamous cell carcinoma (SCC) is a very common form of cancer affecting the squamous cells lining the epithelial layer of organs, primarily the skin and less common in the head and neck regions. SCC grows slowly and may metastasize to other parts of the body making treatment more difficult. SCC often develops on parts of the body exposed to high levels of ionizing UV radiation, such as the skin. 80% of SCC cases of the oral region (tongue, pharynx) are caused by tobacco smoking [1]. In addition to tobacco and alcohol, human papilloma virus (HPV) is associated with a significant proportion of head and neck cancers. As in cervical cancers, HPV types 16 and 18 are the cause of malignant transformation. HPV-positive cancers of head and neck have unique characteristics such as occurrence in a younger age group, distinct clinical and molecular features, and better prognosis as compared to HPV-negative carcinomas [2]

Chronic exposure of the epithelial surfaces to these irritants results in a sequential development from hyperplasia to dysplasia to carcinoma. SCC ranges from well, to moderate, to poorly differentiated grading based on their degree of morphological differentiation and keratinization [1,3]. Well differentiated SCC exhibit abundant cytoplasm, mild atypia, and developed keratinization, whereas poorly differentiated SCC exhibit high nuclear to cytoplasmic ratios and minimal keratinization [1]. Poorly differentiated SCC is difficult to identify using histomorphologic features by light microscopy without additional ancillary studies, such as immunohistochemistry (IHC) studies, which is essential for definitive diagnosis. SCC is often treated by surgical excision, curettage, electrodessication, cryotherapy, and/or radiation therapy [3]. Post-radiation treatments may result in severe cellular atypia making it difficult to distinguish from recurrent squamous cell carcinoma based only on morphologic features. IHC studies can be utilized to make this differentiation. In this report, we propose a flow chart

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providing an algorithm to utilize IHC studies. At the cellular level, radiation therapy can directly affect important target molecules, or indirectly through intermediary radiation products, such as free radicals. These are formed from photons interacting with water [4,5]. Post-radiation therapy causes several cellular changes leading to cell death. The spectrum of these changes may include swollen endothelial cells, telangiectasia of thin walled vessels, nuclear atypia, atypical fibroblasts/myofibroblasts and additional changes in keratinization patterns [5]. These cellular changes are often misdiagnosed as recurring SCC because of the cytomorphologic similarities of radiation-induced atypia and poorly differentiated SCC. IHC stains use labelled antibodies to bind to target antigen in-situ to identify cellular components, with extended utility evaluate diagnosis, stage, grade, cell type, and origin of metastases [6]. The sensitivity and specificity of each IHC marker for a specific cell type vary to a moderate degree. It is essential to consider the frequent IHC mixed reaction for different cell types in providing a specific diagnosis.

Case Presentation

We present a case of a 73-year-old man with a past medical history including poorly differentiated HPV-negative squamous cell carcinoma of the tongue and larynx metastatic to esophagus and lungs and hepatocellular carcinoma treated with combination of surgery, chemotherapy and radiotherapy. Two years following last radiation treatment, the patient presented with a left upper lobe lung mass. FNA of the mass showed scattered groups of highly atypical epithelial cells with high nuclear to cytoplasmic ratios, irregular nuclear membranes with uneven chromatin distribution, and moderate dense cytoplasm. In addition, a second right upper lung nodule was also identified, which showed the same the same histomorphologic features noted in the left lung mass. Histologic assessment of the lung mass with H&E stain shows features highly suspicious for squamous cell carcinoma. However, there was prominent subpleural fibrosis, type II pneumocyte moderate atypia, and metaplastic and vascular changes with organizing thrombus formation (Figure 1 A-C). IHC studies were essential to confirm the diagnosis of squamous cell carcinoma. The atypical cells were negative for P16, P40, P53, Cytokeratin CK5/6, CK7, TTF-1. Some of the atypical cells were positive for CD163 (Figure 1 D) indicating their nature as reactive histiocytes, while other highly atypical cells were positive for the squamous cell marker CD63, but negative for the stronger squamous cell marker P40 indicating their nature as reactive pneumocytes. All cells including the CD163 positive macrophages and the CD63 atypical pneumocytes showed only 3-8 % nuclear staining with the proliferation marker Ki-67 (MIB-1). Due to prior history of hepatocellular carcinoma, Hepar immunohistochemistry marker was utilized to rule out metastasis and was also negative. The IHC studies confirmed the reaction nature of the lesion as a radiation induced atypia and ruled out the morphologic suspicion of squamous cell carcinoma. This supports the utility of IHC in complicated cases to distinguish between recurrent cancer and radiation-induced changes. Patient expired 7 months later due to advanced metastatic disease of his squamous cell carcinoma spreading to the liver, lung, and vertebrae.

Discussion

Distinguishing between SCC and radiation-atypia can be challenging, particularly in poorly differentiated tumours, and should involve the use of immunohistochemical (IHC) markers to avoid diagnostic errors. Radiation induced atypia (RIA) involves changes related to reactive macrophages/histiocytes and reactive pneumocytes. Without the use of IHC studies, RIA can histomorphologically be mistaken for squamous cell carcinoma. To start with, reactive macrophages/histiocytes are positive for CD163, while reactive pneumocytes are negative [7]. At the same time, reactive pneumocytes are positive for P63, but reactive macrophages/histiocytes are negative for the P63 [8]. However, to differentiate between squamous carcinoma cells and reactive pneumocytes, P40 can be used as it is positive in the carcinoma cells but negative in reactive pneumocytes and in reactive macrophages/histiocytes. To further confirm that squamous carcinoma cells are indeed present, both P53 and KI-67 (proliferation marker) can be used [9].

To elaborate on the superiority of P40 over P63, it is first important to understand that P63 is expressed in the basal/progenitor cell layers of stratified epithelia, basal cells of glandular epithelia, and myoepithelial cells of breast and salivary glands [10]. Because of this, p63 can be positive in squamous cell carcinomas, but also in urothelial and myoepithelial neoplasms.

Due to this, P63 can be also positive in adenocarcinomas and lymphomas, making it difficult to differentiate these tumors from squamous cell carcinoma. Therefore, this indicates that p63 has a high sensitivity (100%) but low specificity (60%); it has a PPV of 94.4% but an NPV of 84.4% [11]. This is because p63 is expressed in cells with various isoforms that differ at the N-terminal domain, particularly TAp63 (regulates expression of growth inhibitor genes, serving as a tumor suppressor gene) and DNp63 (antagonizes the activity of TAp63, serving as an oncogene). DNp63 is also considered to be functional as a stem cell factor, promoting regeneration, which is critical in tumor survival and growth [10].

Since there are multiple isoforms of p63, the stain is positive in multiple neoplasms, contributing to its low specificity. However, p40 staining is squamous specific in contrast because this antibody only recognizes the DNp63 isoform. This gives it a sensitivity of 100% but a specificity of 98% [2]. Bishop et al., showed these results as both p63 and p40 were positive in 81/81 of their squamous cell carcinoma specimens. However, p63 also stained positive for 74/237 of the adenocarcinoma cases (31%) while p40 only stained positive for 7/205 cases (3%) [12]. Similarly, p63 showed positive results in 82/152 (54%) of large cell lymphomas, while p40 was negative for all 152 cases [13]. This degree of high specificity for squamous cell carcinomas makes p40 superior to p63 for immunohistochemical staining. Righi et al., found similar results and reported p40 to have a sensitivity and specificity (Figure 2) of 100% and 97%, respectively, for diagnosis of squamous cell carcinomas based on 57 cytological specimens [14].

Based on these results, the diagnosis of squamous cell carcinoma utilizing IHC markers is best to begin with a p40 testing,

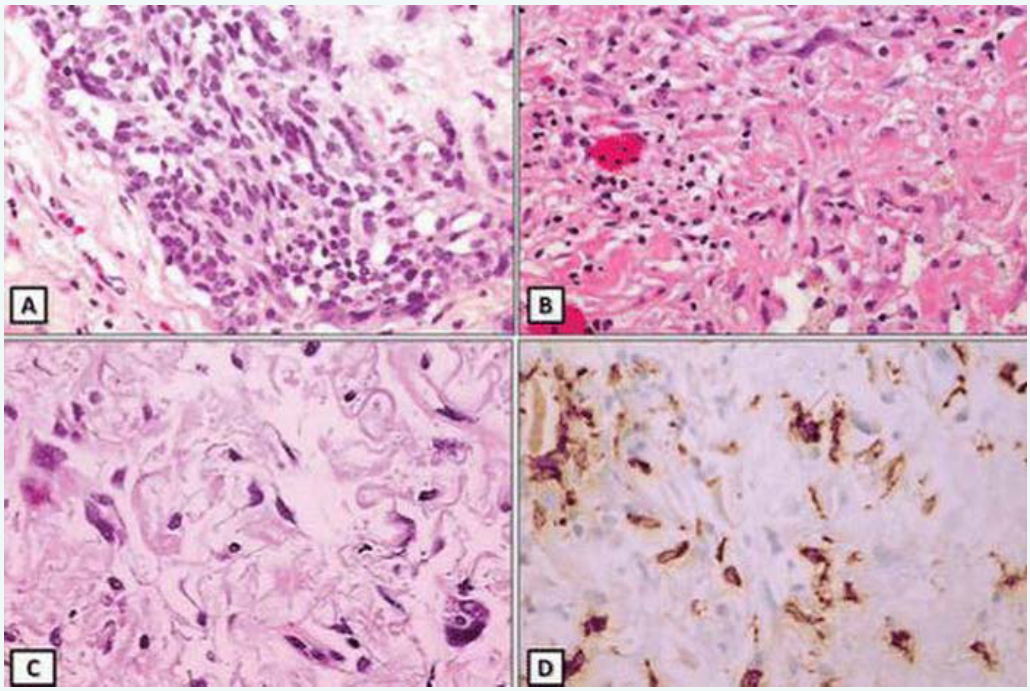

Figure 1 Histologic assessment of the lung mass
A: Sheets, clusters, and single atypical cells highly suggestive of squamous cell carcinoma. H&E stain; X 40
B: Metaplastic and vascular changes with organizing thrombus formation. H&E stain; X 40
C: Reactive macrophages admixed with reactive type II pneumocytes. H&E stain; X 100
D: Immunohistochemistry CD163 positive for the reactive macrophages. Reactive pneumocytes are negative

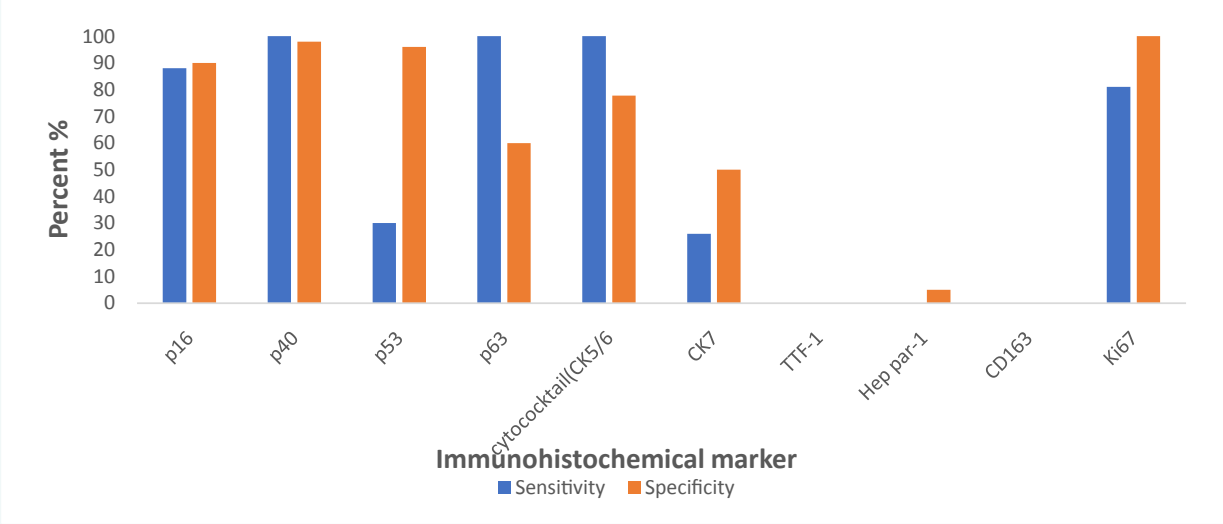

Figure 2 *Graphical representation of the sensitivity and specificity of immunohistochemical markers p16, p40, p53, CytoCocktail (CK5/6), CK7, TTF1, Hep Par-1, CD163, and Ki67.
(* Data collected from prior published reports. See Table-2)

demonstrating 98%-100% specificity and 100% sensitivity on resected specimens from lung SCC [13]. P40 marker's high specificity and a 100% positive predictive value (PPV) (Tables 1,2) lead to highly conclusive results where a positive test rules in SCC with only a 2% margin of misdiagnosis. However, diagnostic error is still possible, and therefore should be followed by IHC CK5/6 as an additional confirmatory marker. CK5/6 has 100% sensitivity, combined with a 100% negative predictive value (NPV), making it effective in ruling out the disease when the test is negative [8]. A combined negative test between p40 and CK5/6

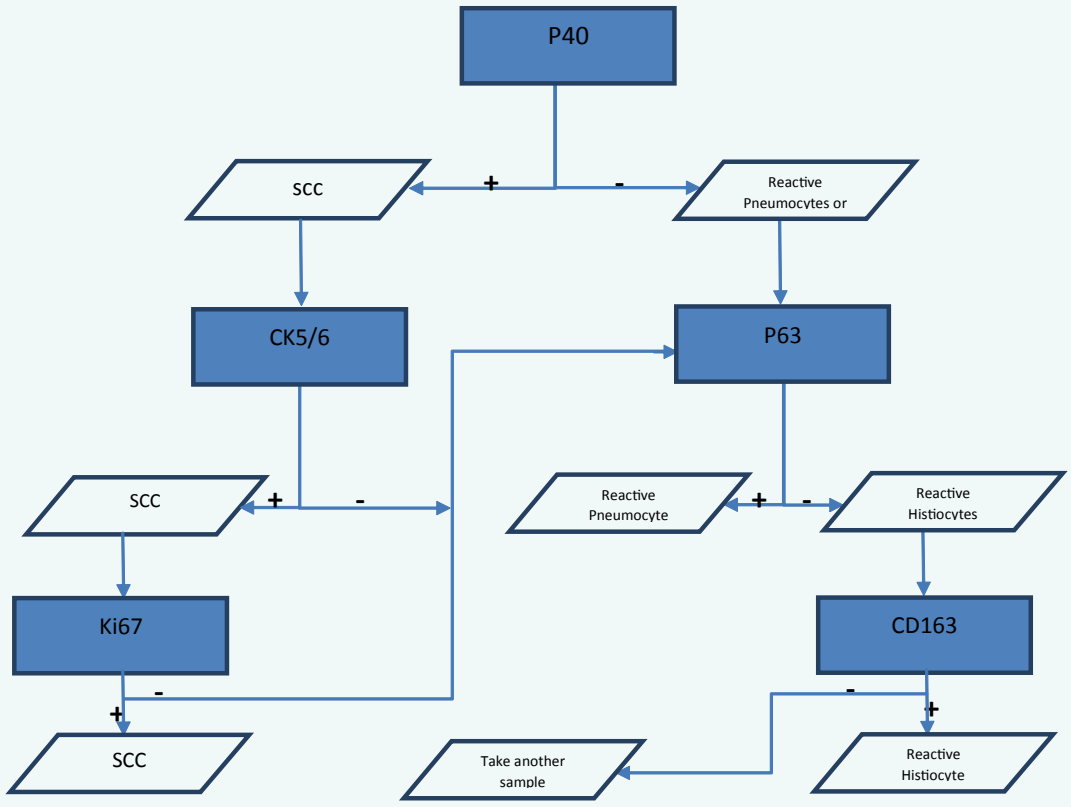

Figure 3 Flow chart devised showcasing a sequential diagnosis of SCC using immunohistochemical markers, p40, p63, CK5/6, CD163, and Ki67 and differentiating it from radiation-induced atypia.
Markers with high diagnostic percentages (specificity, sensitivity, PPV, NPV) are used in the preliminary steps of the flow chart (p40, CK5/6, p633) and lead to further testing with markers having lower percentages (Ki67). A flow of positive and negative tests will help lead to highly-conclusive SCC, weakly conclusive SCC, or radiation-induced atypia. Ki67 is typically used as a confirmation test because of its weak diagnostic values.

Table 1: Percentage sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) diagnostic values of immunohistochemical markers p16, p40, p53, CytoCocktail (CK5/6), CK7, TTF1, Hep Par-1, CD163, and Ki67.

	p16	p40	p53	*P63*	CytoCocktail (CK5/6)	CK7	TTF1	Hep par-1	CD163	Ki67
Sensitivity	88%*	100%	30%	*100%*	100%	26%	n/a **	n/a	n/a	81%
Specificity	90%*	98%	96%	*60%*	77.8%	50%	0%	5%	0%	100%
PPV	38%	100%	86%	*94.4%*	90%	n/a **	n/a **	n/a	n/a	100%
NPV	100%	81.4%	82%	*86.4%.*	100%	n/a **	n/a **	n/a	n/a	89%

(*) Represents percentages retrieved from human papillomavirus induced SCC.
(**) Represents values not found for SCC but have high percentages for differentiating adenocarcinoma.
*** Data collected from prior published reports. See Table 2

is enough to rule out SCC and thus can be assumed to be radiation induced atypia after treatment.

Ki67, a proliferation marker, is found to be exceptional in all statistical values (ranging from 80-100%) and is advised to be used at the end of IHC staining as a test for discerning poorly differentiated SCC [9]. With a specificity of 100% and PPV of 100%, the marker is exceptional at ruling in the disease; however, it can be used after a consecutive number of tests due to its sensitivity of 81% and 86% NPV. Due to such low values, it is not used primarily.

In line with p40 positive test being conclusive in ruling in SCC, P40's 98% high sensitivity and 81.4% NPV is conclusive in ruling out SCC in the event of a negative test and should be followed by CD163, a stain specific for reactive macrophages/histiocytes. This would confirm that the specimen shows evidence of radiation atypia. Unfortunately, there is very little statistical data available

Table 2: Selected previous publications discussing HIS use with SCC and their relevant findings.

Date of Publication	Title	Author	Pertinent findings
1993, July 23	Squamous cell carcinomas. An immunohistochemical study of cytokeratins and involucrum in primary and metastatic tumours.	Suo Z et al. [15]	CK5/6 cocktail resulted in a positive stain for primary tumours in 55% of SCC cases retrieved from the uterine cervix, head and neck, lung, skin, oesophagus, and urinary bladder. The expression of CK 1,4,8,13,18, 19, and 20 were also examined. Majority of SCC expressed CK8 and CK19; and the absence of CK20 staining is helpful in ruling out the disease. The findings show that cytokeratins are a good IHC markers for cancers. The paper does not discuss in what order the IHC markers should be used and only focuses on cytokeratins and involucrin.
2013, May	Immunohistochemistry as an adjunct in the differential diagnosis of radiation-induced atypia versus urothelial carcinoma in situ of the bladder: a study of 45 cases.	Esther et al. [16]	CK20 and P53 negative staining and CD44 positive staining suggest radiation induced atypia contrasted with urothelial carcinoma of the bladder. Although the study discusses urothelial cancer of the bladder, the negative stain of p53 on radiation induced atypia supports our finding that positive p53 staining is a good IHC marker for ruling in SCC. Negative CK20 staining is a good IHC marker for ruling out urothelial carcinoma, which supports the findings of Suo Z et al. where the same diagnosis of CK20 is seen in SCC. The study suggest CK20 to be more reliable than p53, but does not suggest any other IHC markers for ruling in the disease.
2018, Mar 14	Update on Immunohistochemistry for the Diagnosis of Lung Cancer	Kentaro Inamura [17]	IHC markers that result in a positive stain for ruling in SCC include p40, CK5/6, and p63. P63 positive staining is not unique to SCC (100% positive) because adenocarcinoma is also positive for P63 in 31% of cases and so was not considered in our research. TTF-1 sensitivity and specificity is not reliable because they are different in the major clones of TTF-1, SPT24 and 8G7G3/1 (17%, 80% and 1%, 70% specificity and sensitivity respectively. Our research concluded TTF-1 to be a very weak IHC marker for TTF-1, which coincides with the pitfall in its different diagnostic values respective to the TTF-1 clone. The study only focuses on ruling in the disease using IHC markers p40, CK5/6, and P63 but does not include markers effective in ruling out the disease.

on the usage of CD163, however, with a negative P40, KI67, P63, and positive CD163, this can serve as strong evidence of radiation atypia.

Conclusion

In this report, we present a simple algorithm to utilize immunohistochemistry studies to differentiate the diagnostically challenging radiation atypia from recurrent squamous cell carcinoma. This is a crucial differentiation, which is essential for optimal management plans. It is our hope that this report raises awareness of surgical pathologists to this challenging differentiation, and that continued investigation drives further development of efficacious diagnosis and safe treatments for improving patient outcomes.

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