Markers for Sebaceoma Show a Spectrum of Cell Cycle Regulators, Tumor Suppressor Genes, and Oncogenes

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Abstract

Background: Sebaceoma is a tumor for which the causative oncogenes are not well-understood. Sebaceomas demonstrate some histopathologic features similar to basal cell carcinoma (BCC), such as palisading borders and basaloid cells with additional features, including foamy cytoplasm and indented nuclei. **Aims:** We examine multiple cell-cycle, oncogene, and tumor suppressor gene markers in sebaceomas, to try to find some suitable biological markers for this tumor, and compare with other published studies. **Materials and Methods:** We investigated a panel of immunohistochemical (IHC) stains that are important for cellular signaling, including a cell cycle regulator, tumor suppressor gene, oncogene, hormone receptor, and genomic stability markers in our cohort of sebaceomas. We collected 30 sebaceomas from three separate USA dermatopathology laboratories. The following IHC panel: Epithelial membrane antigen (EMA)/CD227, cytokeratin AE1/AE3, cyclin D1, human breast cancer 1 protein (BRCA-1), C-erb-2, Bcl-2, human androgen receptor (AR), cyclin-dependent kinase inhibitor 1B (p27^{kip1}), p53, topoisomerase II alpha, proliferating cell nuclear antigen, and Ki-67 were tested in our cases. **Results:** EMA/CD227 was positive in the well-differentiated sebaceomas (13/30). Cyclin-dependent kinase inhibitor 1B was positive in tumors with intermediate differentiation (22/30). The less well-differentiated tumors failed to stain with EMA and AR. Most of the tumors with well-differentiated palisaded areas demonstrated positive staining for topoisomerase II alpha, p27^{kip1}, and p53, with positive staining in tumoral basaloid areas (22/30). Numerous tumors were focally positive with multiple markers, indicating a significant degree of variability in the complete group. **Conclusions:** Oncogenes, tumor suppressor genes, cell cycle regulators, and hormone receptors are variably expressed in sebaceomas. Our results suggest that in these tumors, selected marker staining seems to correlate with tumor differentiation; that is, well-differentiated tumors as a group stained with EMA and AR, and palisaded areas demonstrated consistent p53, topoisomerase II alpha and p27^{kip1} staining. In contrast, less well-differentiated areas stained with a different spectrum of markers.

Keywords: Cell cycle regulators, Sebaceoma, Tumor suppressor genes and oncogenes

Introduction

Sebaceoma is a clinically indolent tumor that should be distinguished from basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and sebaceous carcinoma.[1-4]

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Sebaceoma is nosologically difficult to classify vis-a-vis other benign and malignant sebaceous neoplasms. To diagnose a sebaceoma, the tumor must display some degree of sebaceous differentiation within tumor lobules, and additional basaloid differentiation in the periphery of the lobules.[1-4] The majority of morphological studies classifying these tumors have used hematoxylin and eosin (H and E) data. Occasional studies have utilized immunohistochemical (IHC) markers.[3-5] We attempted to evaluate a group of these tumors utilizing a panel of IHC markers, including cytokeratin AE1/AE3, epithelial membrane antigen (EMA), and multiple cell-cycle oncogene and tumor suppressor gene markers. We

acknowledge that this study is limited by relatively small number; however, this entity is uncommon and thus our findings will lend some insight to the biologic and/or diagnostic classification of sebaceomas.

Materials and Methods

H and E staining

We studied 30 archival skin biopsies with independent diagnoses of sebaceoma from three dermatopathology laboratories; each biopsy diagnosis was performed by a board certified dermatopathologist in the USA. The study was excepted from Institutional Review Board requirements because the samples were without patient identifiers as well as archival biopsies. The parameters such as age, sex, and race of the subjects for the 30 biopsies were also analyzed. All biopsies were initially fixed in 10% buffered formalin, embedded in paraffin, and cut at 4 micron thicknesses. The tissues were stained with H and E as previously described.[6-8] Our study included only lesions that had typical features of basaloid, palisading margins, and the presence of cells with some degree of sebaceous differentiation inside the tumor.[1-4] Tumors with i) a majority of sebaceous cells demonstrating severe cytologic atypia, or ii) high sebaceous component mitotic rates (focally greater than four mitotic figures per high powered, ×400 microscopic field) were excluded and considered to be sebaceous carcinomas.

IHC staining

The IHC staining was read as positive or negative, in the presence of a negative and a positive control for each marker tested. These readings were performed by an immunodermatologist and were based on the positivity of the stain/mm^2 of tumor, at ×200 and ×400 total magnification. Our IHC staining was performed as previously described.[6-8] The extent and location of IHC staining for each marker was assessed according to an immunoreactive score (IRS) that evaluated the proportion of cells expressing each marker and the intensity of staining. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); and the percentage of positive cells examined was scored as 0 (negative staining), 1 (<10%), 2 (11-50%), 3 (51-80%), and 4 (>80%). The two scores were then multiplied together and the IRS (values from 0-12) was determined: 0 as negative, 1-3 as weak, 4-6 as positive, and 7-12 as strongly positive. No quantitative or semiquantitative results with regard to proliferative markers were done. For IHC, we utilized the following Dako antibodies: Monoclonal mouse anti-human EMA/CD227, monoclonal mouse anti-human p53, monoclonal rabbit anti-human cyclin D1, monoclonal mouse anti-human breast cancer type 1 susceptibility protein (BRCA-1), proliferating cell nuclear antigen

(PCNA), monoclonal mouse anti-human Ki-67 antigen, polyclonal rabbit anti-human C-erB-2 oncoprotein, (also called HER2/neu, human EGF receptor 2, and human epidermal growth factor receptor 2), monoclonal mouse anti-human cyclin-dependent kinase inhibitor 1B (p27^{Kip1}), monoclonal mouse anti-human androgen receptor (AR), monoclonal mouse anti-human topoisomerase II alpha, and monoclonal mouse anti-human cytokeratin AE1/AE3. Clone AE1 detects the high molecular weight cytokeratins 10, 14, 15, and 16, and also the low molecular weight cytokeratin 19. Clone AE3 detects the high molecular weight cytokeratins 1, 2, 3, 4, 5, and 6. All tumors were stained with each marker and positive and negative controls were performed for each stain.

Statistical analysis

For statistical analysis, we utilized the nonparametric Mann-Whitney U-test to calculate significant levels for all measurements including the standard deviation (SD). Values of $P < 0.05$ were considered statistically significant.

Results

From 30 skin biopsies, 17 were from males and 13 from females with a mean age at resection of 60 ± 10 years. Several cellular proliferation markers (c-erb-2, Ki-67, Bcl-2, and Cyclin D1) were focally positive in areas inside the tumors, and also in epidermal areas superjacent to the sebaceomas $P < 0.05$. The majority of the well-differentiated sebaceomas stained for EMA/CD227 within the tumor, with $P < 0.05$ (13/30; significantly more staining than in less well-differentiated parts of the tumors). Many tumors were stained by all of the markers; however, EMA was only positive in the well-differentiated ones [Table 1 and Figure 1]. For each antibody staining pattern, please refer to Table 1 for a description of the pattern, as well as respective IRS scores. Basaloid, less well-differentiated areas of the tumors consistently failed to stain with EMA, but were consistently positive with topoisomerase II in areas with a palisading histology $(P < 0.05)$. In these areas, we noted significantly more topoisomerase II staining than in well-differentiated parts of the sebaceomas (24/30). Topoisomerase II was positive in four different patterns among the tumors [Table 1]. PCNA was positive only in 9/30 of the less well-differentiated sebaceomas. In some sebaceomas, we noted mixed patterns, with basaloid cells admixed with areas of sebaceous differentiation and squamous differentiation; the squamous differentiation was highlighted by cytokeratin AE1/AE3 expression. Both the AE1 and AE3 individual clones detect specific high and low molecular weight keratins [Table 1]. In addition,

Table 1: Markers for sebaceoma show a spectrum of cell cycle regulators, tumor suppressor genes, and oncogenes

Marker	Role	Positive staining cases, and IRS values	Notes
P53, tumor protein p53, cellular tumor antigen p53, phosphoprotein p53, or tumor suppressor p53, is a protein that in humans is encoded by the TP53 gene	Tumor suppressor gene that regulates the cell cycle. The p53 protein is critical in multicellular organisms where it regulates the cell cycle, and functions as a tumor suppressor preventing cancer	(SD 1.36), IRS 10-12, (range: 1-30)	p53, with positive staining in tumoral basaloid areas and in the periphery of the basaloid areas of the epithelial component of the tumor. Positivity was also seen in the normal epithelia above the tumor
Topoisomerase II	Participates in checkpoint activation, and in the maintenance of genomic stability. Topoisomerase II is an essential enzyme that plays a role in virtually every cellular DNA process	(SD 1.25), IRS 4-6, (range: 1-30)	Four types of staining were seen, one in the palisaded areas (well-differentiated areas of the tumor). A second pattern in the extracellular matrix cells around the tumor. A third pattern, resembling stem cells, intermittent in the tumor. The fourth pattern of positivity was seen at the basement membrane zone (BMZ) of apparently normal skin above the tumor
Bcl-2 (B-cell lymphoma 2), encoded in humans by the Bcl-2 gene	Apoptosis regulator. Bcl-2 is the part of the member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptosis), or inhibiting it (antiapoptotic). Bcl-2 is an oncogene	(SD 1.57), IRS 4-6, (range: 1-30)	Positive in a pattern of intermittent cells, mostly around tumor cells with basaloid differentiation. Another pattern was positive staining in the area of hair follicle, where a new tumor seemed to be forming in basement membrane areas
P27^{Kip1}, also known as kinase inhibitory protein 1 (Kip1)	Cell cycle regulator inhibitor, that binds to cyclin-dependent kinase (CDK) cyclin E/CDK2 complexes, but not to CDK2 alone. p27 is a CDK inhibitor, acting in G1 to inhibit cyclin-CDKs. As negative growth regulators, the CDK inhibitors may function as tumor suppressors	(SD 1.26), IRS 10-11, (range: 1-30)	Four major patterns of staining were seen. The first in the sebaceous cell, partially or well-differentiated areas of the tumor). The second in extracellular matrix cells around the tumor. The third in the epidermis above and/or around the tumors. The fourth in palisaded, basaloid areas of the tumor
BRCA-1 (breast cancer 1, early onset)	Helps repair damaged DNA, or destroy cells if DNA cannot be repaired. BRCA1 is a human tumor suppressor gene that protects normal DNA	(SD 3.75), IRS 4-7, (range: 1-30)	Positive in those tumors with basaloid, epithelioid, and myxoid components, especially those more poorly differentiated
Cyclin D1	Cell cycle regulator. Cyclins function as regulators of progression of the cell cycle. Cyclins function as regulators of CDK. Different cyclins exhibit distinct expression and degradation patterns, which contribute to the temporal coordination of each mitotic event	(SD 1.86), IRS 5-7, (range: 1-30)	Positive staining, mostly seen in a single pattern. Was seen in basaloid areas, where the tumors were originating from the epidermis. The majority of the tumors positive for this marker were also positive for P53
Ki-67 or MKI67	A marker for cell proliferation. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0)	(SD 1.2). IRS 5-7, (range: 1-30)	Positive in a pattern of individual, intermittent cells, mostly around cells with basaloid differentiation. Also positive in areas of apparently normal epidermis near the tumors
C-erB-2, also called HER2/neu, human EGF receptor 2, and human epidermal growth factor receptor 2.	A hormone receptor tyrosine kinase. A protein involved in normal cell growth; however, it is also found on some types of cancer cells	(SD 3.3), IRS 5-10, (range: 1-30)	Positive in the cytoplasm of the palisaded parts of some tumors, mainly in less differentiated tumors
EMA/CD227. Also termed MUC1 and episialin	EMA/CD227 is a cell surface mucin, originally thought to be restricted to epithelial tissues	(SD 2.3). IRS 10-12, (range: 1-30)	Staining was primarily noted in well-differentiated tumors, especially those originating from the epidermis. In addition, EMA was ectopically expressed in upper layers of the adjacent epidermis in the majority of the tumors that were positive for this marker

Continued

Table 1: (Continued)

Marker	Role	Positive staining cases, and IRS values	Notes
Androgen receptor, also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4)	A hormone receptor. Specifically, a type of nuclear receptor that is activated by binding of either of the androgenic hormones testosterone or dihydrotestosterone in the cytoplasm and then translocating into the nucleus	(SD 3), IRS 3-7, (range: 1-30)	Staining was noted in well-differentiated, palisaded areas
Proliferative cell nuclear antigen (PCNA)	PCNA is a cell cycle regulator, and plays important roles in DNA synthesis and DNA postreplication repair	(SD 3.3), IRS 5-8, (range: 1-30)	Positive, focal staining was noted in some of the most undifferentiated tumors
Cytokeratin AE1/AE3	Recognizes Moll cytokeratins 10, 14, 15, 16, 19, 1, 2, 3, 4, 5, 6, 7, 8	(SD 2.3), IRS 10-12, (range: 1-30)	Three positive staining patterns were seen. The first was focal staining, in multiple differentiation areas of the same tumor. The second was in tumor basaloid/epithelioid areas. The third was noted in extracellular dermal areas and in the epidermis around the tumor

EMA = Epithelial membrane antigen, DNA = Deoxyribonucleic acid, SD = Standard deviation, IRS = Immunoreactive score

a subset of our sebaceomas displayed a staining overlap with BCCs, based upon the observed positivity of p27^{kip1}, p53, and topoisomerase II alpha markers in the basaloid cells of the sebaceomas [Figure 1]. In comparison to the other sebaceomas in our series, the observed positive staining for these markers was statistically significant, with $P < 0.05$. Human AR was only positive in well-differentiated sebaceomas [Table 1]. The majority of our biopsies were completely negative for C-erB-2 oncoprotein, BRCA-1, and Ki-67.

Discussion

The H and E histologic diagnosis of sebaceoma is challenging and IHC markers have been previously utilized to assist in the diagnosis.[1-3,9,10] Our results similarly identified high variability among sebaceomas with regard to their expression of markers of i) cell cycle regulators, ii) oncogenes, iii) tumor suppressor genes, and iv) related proteins and hormones receptors, suggesting an immunologic spectrum of tumors defined as sebaceomas.

We identified expression of the majority of these markers in our cohort of sebaceomas. The expression of many of the markers was observed within the tumors and interestingly, also in the surrounding tumoral microenvironment including the overlying epidermis. Several tumors were demonstrated with variegated expression in foci within the tumor, and also surrounding the tumors. These two findings may be indicative of a significant immunologic variability among sebaceomas, as well as the possibility that the tumors contribute variably to immunologic changes in their surrounding microenvironment. This microenvironment expression data may prove useful, since tumoral microenvironment

alterations are becoming increasingly important in the setting of personalized therapy. We found diverse degrees and patterns of positivity for specific markers across multiple cases, reinforcing these concepts. Our study has suggested that not all sebaceoma cells are identically altered, accounting for the variability of staining in each tumor. Pertinently, aneuploidy (an atypical number of chromosomes) is a trait common to most solid tumors.[11]

PCNA expression is found within many essential cellular processes, such as deoxyribonuclease acid (DNA) replication, repair of DNA damage, chromatin structure maintenance, chromosome segregation, and cell-cycle progression. Overexpression of cyclin D1 expression has been observed frequently in a variety of tumors, such those described here.[12] p27^{Kip} is known to be involved in regulation of cell proliferation, and may contribute to the tumor genesis seen in sebaceomas. Moreover, studies in several tumor types indicate that p27^{Kip} expression levels have both prognostic and therapeutic implications in several tumors.[12] We are conducting further studies with this marker; our preliminary results show that p27^{Kip} is a proliferation marker that tends to be positive where proliferating sebaceous cells are present.

BRCA-1 is a human gene that produces a tumor suppressor protein. The BRCA-1 protein helps repair damaged DNA, and therefore plays a role in ensuring the stability of the cell's genetic material. When the BRCA-1 gene is mutated or altered, such that its protein product is not made or does not function correctly, damaged DNA may not be repaired properly. As a result, cells are more likely to develop additional genetic alterations that can lead to cancer. For the markers we explored, is difficult to know if the body is
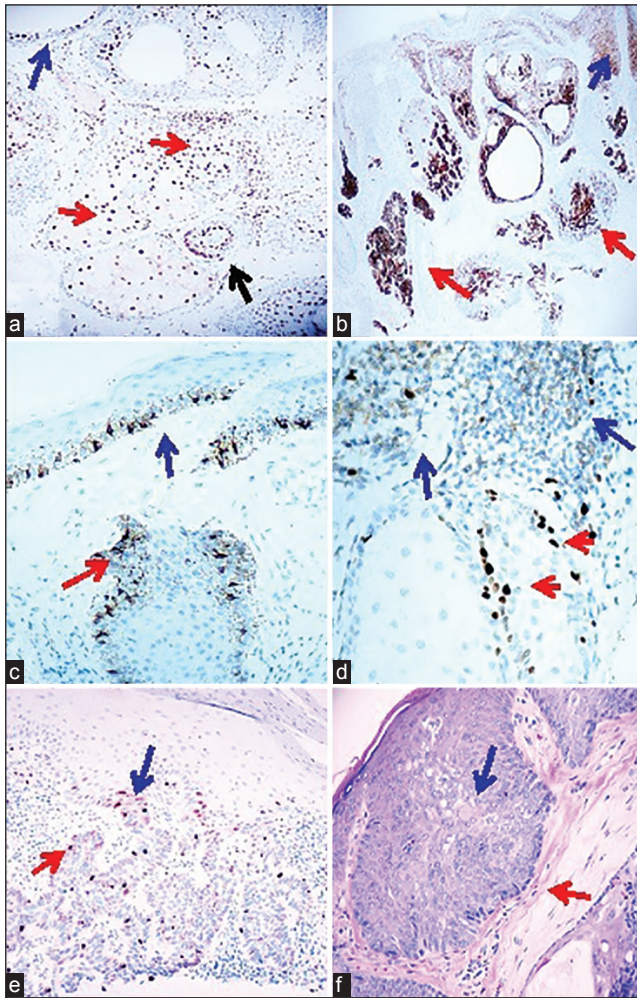
Figure 1: Immunohistochemical staining panel on sebaceoma. **a.** IHC, with P27Kip positive staining inside a tumor (brown staining; red arrows), and also in the adjacent epidermis above the tumor (brown staining; blue arrow) (100×) (the black arrows is positive around the tumor. **b.** IHC, demonstrating EMA/CD227 positive staining in a tumor (brown staining; red arrows) and also in the suprajacent epidermis (brown staining; blue arrow) (40×). **c.** IHC, demonstrating PCNA in the normal tissue above the tumor and in the bulb origin from the epidermis, positive staining inside a tumor palisade areas (brown staining; red arrow), and in the suprajacent epidermis (brown staining; blue arrow) (200×). **d.** IHC staining with Topoisomerase II, positive inside a tumor in palisaded areas (red arrow) and in surrounding peritumoral areas (brown staining; blue arrow) (400×). **e.** Cyclin D1 IHC staining, positive in palisaded areas of a tumor (brown staining; red arrow) as well as in an area where the tumor seems to be originating from the epidermis (brown staining; blue arrow) **f.** H&E staining of a tumor demonstrates focal features of BCC, with the palisaded areas (red arrow) and sebaceous differentiation areas (blue arrow).

already responding to the tumor via tumor suppressor genes, and thus attempting to neutralize any genomic instability.[10,12] Our exploratory study is limited by its sample size; however, our study demonstrates that sebaceomas express different IHC markers depending on their degree of differentiation. We conclude that

sebaceomas display abnormal expression of several oncogenes, tumor suppressor genes, cell cycle regulators, and hormone receptors depending of the degree of differentiation. Further molecular characterizations and correlations with the degree of differentiation of the tumors are warranted. In this context, we are correlating our data with further research on BCCs and SCCs, with the aim of determining a diagnosis, and further specific information on tumor differentiation based on the altered expression of oncogenes, cell cycle regulators, and tumor suppressor factors.

Further suggestive studies may include comparison of sebaceoma with sebaceous adenoma and with sebaceous carcinoma and the presence of oncogenes, tumor suppressor genes, and cell cycle regulators.

Acknowledgments

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