Mechanistic and Treatment Implications of ΔNp63 Expression in a Rare Case of Metastatic Hidradenocarcinoma

James J. Driscoll, MD, PhD
Medical Oncology Branch, National Cancer Institute
Building 10–12N226, 10 Center Drive
Bethesda, MD 20892-1906 (USA)
Tel./Fax +1 301 443 5397, E-Mail driscollj@mail.nih.gov

Steven Gauerke, MD
Department of Anatomic Pathology, National Naval Medical Center
Bethesda, MD, USA

Brian C. Monahan, MD
Department of Pathology, F. Edward Hebert School of Medicine
Uniformed Services University of the Health Sciences
Bethesda, MD, USA

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Abstract
Hidradenocarcinomas are rare, aggressive adnexal tumors of sweat gland origin that demonstrate a high potential for local recurrence, metastasis and poor outcome. These neoplasms can derive from preexisting clear cell hidradenomas, but more commonly appear de novo with the molecular events responsible for the pathogenesis currently unknown. Molecular markers of pathogenesis as well as effective forms of adjuvant chemotherapy are missing due to the lack of accurate diagnosis, paucity of cases and confusion with other visceral solid tumors. Here, we report a 37-year-old man who presented with a rapidly growing, painful palpable mass located in the right inguinal area. The patient was a nonsmoker, did not consume alcohol and had a medical history remarkable only for a lower abdominal superficial skin lesion in the same area that had been excised 11 years earlier. Although initially slow growing, the lesion eventually expanded, was surgically excised and was diagnosed as a hidradenoma. There was no family history of malignancy and the patient had not experienced any constitutional symptoms. We probed the immunohistochemical status and detected negative staining for the estrogen, progesterone and Her2 receptors, while strong, diffuse nuclear staining was seen in the majority of cells consistent with p53 overexpression. Similarly, strong nuclear reactivity was seen with p63 and p73 antibodies. The p63 gene contains 2 separate promoters which express at least 6 major transcripts that lead to 2 fundamentally different classes of proteins; 3 isoforms (TAp63α, β and γ) encode proteins that induce apoptosis, whereas the other 3 isoforms (ΔNp63α, β and γ) may exert inhibitory effects on p53. Interest in p63 stems from this 'two genes in one'-concept. Importantly, the nuclear presence of ΔNp63 was detected widespread throughout the tumor. We have identified a subtype of hidradenocarcinomas that express ΔNp63 and
uncovered an unforeseen commonality with triple-negative breast tumors. To our knowledge, this is the first report of a sweat gland tumor that displayed expression of both ΔNp63 and p73 and demonstrated a triple-negative receptor status. Such a link between 2 seemingly disparate tumor types indicates a mutual pathway of tumorigenesis and suggests the potential for common therapeutic regimens.

Background

Hidradenocarcinomas are rare, intradermal malignant sweat gland tumors that present as asymptomatic, nondescript cutaneous lesions with a reported incidence rate of around 0.05% in the United States [1]. Diagnosis is made primarily in the fifth to seventh decades of life, with similar incidence in men and women and without racial predilection. Historically, diagnosis has been difficult due to the paucity of cases, inconsistent nomenclature, variable morphology and confusion with visceral, metastatic tumors. Generally, lesions present on the face or extremities; however, cases have also been reported with lesions on the abdomen, trunk and groin and even more unusually on the scalp, elbow and digits. Typically the tumor appears as a 'benign' solitary skin lesion and maintains a stable size or may slowly expand. Hidradenocarcinomas exhibit a lack of distinguishable features and following excisional biopsy or local excision, the primary lesion may remain dormant or recur slowly in situ. Most patients remain asymptomatic without any overt effect other than pain, bleeding with contact or ulceration. At some point, through an unknown mechanism, the tumor assumes an aggressive clinical course with growth at regional or distant metastatic sites; primarily lymph nodes. Tumors demonstrate up to 50% local recurrence despite aggressive surgical management, and prognosis is generally poor with a 5-year disease-free survival rate of less than 30% [2].

Case Presentation

A 37-year-old Caucasian male presented to his internist to report an enlarged, tender right inguinal mass that had progressively increased in size over the previous months. In addition, he noted a long-standing, smooth, quarter-sized skin lesion on his right lower abdomen which had recently begun to bleed at the base upon physical contact. Eleven years earlier, the same skin lesion had been biopsied at an overseas medical facility and was diagnosed by gross pathology as a hidradenoma that extended to the surgical margins with recommendation for complete excision. Shortly after biopsy the mass recurred, elevated the surrounding skin and tripled in size. The lesion remained otherwise stable and the patient was lost to follow-up for over a decade. Upon presentation, physical examination revealed an otherwise healthy, age-appropriate male, without any other palpable lymphadenopathy or organomegaly. A complete blood count and comprehensive metabolic panel were unremarkable and a viral screen was negative. Subsequent to presentation, both the abdominal lesion and right inguinal lymph node were concurrently excised.

Gross Pathology and Microscopic Findings

The gross abdominal specimen measured 4.2 × 2.5 cm, was excised to a depth of 3.2 cm, demonstrated an elevated, ulcerated surface and was comprised of atypical, uniform polygonal cells suspicious for malignancy of adnexal origin (fig. 1a). At high power, residual multinodular tumor was observed with infiltrative growth which consisted of epithelial cells with clear cytoplasm, round nuclei, pleomorphism, atypia and few mitoses (fig. 1b) These morphologic features were consistent with those of a hidradenocarcinoma. Many of the glandular lumen demonstrated decapitation structures which indicate the release of part of the apocrine cell into the central part of the gland. The right inguinal lymph node was then radically resected with the capsule intact, measured 3.5 × 2.5 × 1.5 cm and exhibited homogenous, dull, tan tissue with punctate areas of hemorrhage (fig. 1c). The lymph node
tissue was nearly entirely replaced by tumor and was composed of similar epithelial cells with clear cytoplasm, ductal structures, infiltrative growth, atypia and mitoses. There was no evidence of geographic necrosis.

**Immunohistochemistry**

Tissue from the lymph node was then probed with antibodies directed against cytokeratins, the p53 tumor suppressor family, the apoptotic regulator Bcl-2, the epidermal growth factor receptor (EGF-R) and the estrogen (ER), progesterone (PR) and Her2 receptors which define distinct breast cancer subtypes (Table 1). Tissue stained strongly for CK7, but was negative for CK20 consistent with a simple (glandular) epithelial origin. An antibody that recognized all known p53 isoforms (pan-53 antibody) yielded strong and diffuse nuclear staining in the majority of cells consistent with p53 overexpression (fig. 2a). Similarly, when probed with a pan-p63 antibody, a majority of cells also demonstrated strong nuclear reactivity and, importantly, these immunoreactive cells were seen throughout the tumor (fig. 2b). Normally, p63 is expressed in the progenitor cell layers of certain tissues, e.g., skin, breast and prostate [3, 4], but these basal cells lose p63 expression as the cells withdraw from the stem cell compartment [5]. In transfected human keratinocytes, in vitro studies have demonstrated that p63 triggers differentiation and is then down-regulated in terminally differentiated cells [3–5]. Moreover, p63 isoforms were then shown to function collectively as the molecular switch which regulates the developmental program of stratified epithelia [5]. A monoclonal antibody directed against the third member of the p53 family, p73, also demonstrated extensive, nuclear reactivity in the majority of cells in contrast to that seen in normal tissue (fig. 2c).

A role for p63 in tumorigenesis has been implied by genomic amplification as well as overexpression in primary squamous cell tumors of the head and neck, lung, bladder, salivary gland and a subset of breast tumors [7–10]. The p63 gene contains 2 separate promoters which express at least 6 major transcripts that lead to 2 fundamentally different classes of proteins [2] (fig. 3). Three of the p63 isoforms (TAp63α, β and γ) encode proteins with roles similar to p53, i.e., transactivation and induction of apoptosis, whereas the other 3 isoforms (ΔNp63α, β and γ) lack the acidic amino-terminal transactivation domain and may exert inhibitory effects on p53 activity. The ΔNp63 forms may function in a dominant negative manner to inhibit p53 by impairing tetramerization, blocking DNA binding or by physically binding to their gene products to inhibit functional activity. Aberrant ΔNp63 expression may preclude apoptosis-inducing activity and maintain the proliferative capacity of basal/progenitor cells. Interest in p63 stems from this ‘two genes in one’-concept, where differential transcription of a single gene yields products with either agonist or antagonist roles involved in neoplastic transformation. Importantly, the nuclear presence of ΔNp63 was detected widespread throughout the tumor (fig. 2d). Finally, weak staining was detected using an anti-EGF-R antibody, while no labeling was seen with antibodies directed against Bcl-2 or S-100 and, importantly, a lack of ER, PR and Her-2 staining was demonstrated which paralleled the subset of triple-negative breast cancers (data not shown).

**Molecular Pathogenesis**

To our knowledge, this is the first case report of a sweat gland tumor that displayed expression of both ΔNp63 and p73 and in addition demonstrated a triple-negative receptor status. A case of metastatic hidradenocarcinoma with ER/Her-2 status by IHC was reported and suggested a responsiveness to tamoxifen or trastuzumab [11]. In addition, a tenuous connection has been reported between Her-2 positivity and adnexal neoplasms [12]. Therefore, we explored the association between these breast tumor markers and adnexal neoplasms in order to elucidate the pathologic events responsible for tumorigenesis and to identify targeted forms of therapy. Global gene expression profiling uncovered previously unrecognized subsets of human breast cancer that include the triple-negative tumors characterized by the lack of ER and PR expression, the absence of Her2 amplification, and the expression of basal epithelial markers [13]. Triple-negative breast cancers are the most common subtype arising in patients that harbor BRCA1 germline mutations. Recently, it was demonstrated that the ΔNp63 and TAp73 isoforms were coexpressed exclusively within certain triple-negative primary breast cancers that commonly exhibited p53 mutational inactivation. Consequently, inhibition of p63 expression by RNA interference led to TAp73-dependent induction of Bcl-2 family members and subsequent apoptosis. Furthermore, breast cancer cells expressing ΔNp63α and TAp73 exhibited sensitivity to cisplatin, but not to other chemotherapeutic agents. Clearly, in tumors as rare as hidradenocarcinomas gene expression profiling is less feasible. However, by immunohistochemical methods, we have demonstrated the overexpression of p53, p63 and p73, the lack of ER and PR expression and the absence of Her2 amplification. Overexpression of the p53 protein predicts a mutated
gene status in this tumor type with a resultant effect on stability and functionality. In addition, we demonstrate the overexpression of ΔNp63 and expand the spectrum of tumors previously identified to exhibit deregulation of the p63/p73 pathway to include sweat gland tumors.

**Treatment and Management**

Currently, treatment options for sweat gland carcinomas are limited and surgical excision, with wide resection margins and regional lymph node dissection, is the only known curative therapy for patients with localized disease and, thus, was used as the primary treatment of this patient. Established guidelines that demonstrate a survival benefit for adjuvant chemotherapy in patients with newly diagnosed or recurrent disease are lacking, in part owing to the rarity of the disease and because large, randomized trials have not been performed. Similarly, the efficiency of adjuvant chemotherapy in combination with radiotherapy has not been demonstrated. Moreover, successful or transient response to various chemotherapy agents is limited to isolated case reports. Owing to the benefit of capecitabine in breast and colon cancer, a single hidradenocarcinoma patient was treated with this agent and demonstrated greater than 50% clinical remission with acceptable clinical and biological tolerance [14]. Such a promising objective response in a single patient suggests further study. Other case reports in patients with measurable disease included less successful treatment that used bleomycin with vincristine, vincristine with cyclophosphamide and vincristine combined with actinomycin D, VM-26, doxorubicin and dacarbazine [15]. The patient described in our report remains in complete remission 12 months following diagnosis and excisional biopsy. In addition, the patient deferred on systemic chemotherapy in part because of the current lack of measurable disease.

**Conclusions**

The identification of novel cancer subtypes may facilitate more accurate diagnoses and promote the development of more specific, more effective and less toxic therapies for even rare tumors such as hidradenocarcinomas. Since a subset of breast tumors are triple negative and invoke the p63/p73 pathway with implications for cisplatin-based treatment, and since they are so rare and so little is known regarding the molecular events that are responsible for tumorigenesis, we explored the status of this tumor. By such an approach, we have identified a subtype of hidradenocarcinomas that harbor ΔNp63 and have uncovered an unforeseen commonality with the triple-negative breast tumors. Such a link between 2 seemingly disparate tumor types indicates a mutual pathway of tumorigenesis and suggests the potential for common therapeutic regimens. A role for the ΔNp63/TAp73 pathway may extend beyond primary breast cancers since ΔNp63α overexpression has been reported in numerous other squamous cell cancers [5–9]. Our findings support a role for ΔNp63 as a survival factor in certain metastatic adnexal skin cancers similar to its role in other solid tumors, and they provide a basis for treatment of metastatic disease with cisplatin-based regimens and indicate that these molecular markers may serve as a useful clinical predictor of cisplatin-sensitivity in treatment-refractory tumors. Finally, the further development of molecular signatures remains of paramount importance to further unravel the biology specific to individual tumor subtypes and to guide the early clinical development of solid tumor treatment strategies.

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**Table 1.** Immunohistochemical profile of metastatic hidradenocarcinoma tissue

| Molecular marker | Staining         | Subcellular localization   |
|------------------|------------------|----------------------------|
| 1. CK7           | positive         | cytoplasmic                |
| 2. CK20          | negative         | –                          |
| 3. p53           | positive         | nuclear                    |
| 4. p63           | strongly positive| nuclear                    |
| 5. p73           | positive         | nuclear                    |
| 6. ΔNp63         | strongly positive| nuclear                    |
| 7. EGF-R         | very weakly positive | cell membrane           |
| 8. Bcl-2         | negative         | –                          |
| 9. S-100         | negative         | –                          |
| 10. ER           | negative         | –                          |
| 11. PR           | negative         | –                          |
| 12. Her-2        | negative         | –                          |

Antibodies used to stain the metastatic hidradenocarcinoma tissue were: CK7 (Dako M7018), CK20 (Dako M7019), p53 (Ventana D0–7), p63 (Dako M7247), p73 (Zymed 32–4200), ΔNp63 (Biolegend 619001), Bcl-2 (Dako N1587), S-100 (Dako Z-0311), EGF-R (Dako M7298), ER (Dako M7047), PR (M3569) and Her2 (Dako K5204).
Fig. 1. a Hematoxylin-eosin staining of skin tumor tissue with gland formation, hyaline deposition and irregular, deep penetration (original magnification ×20). b High power hematoxylin-eosin view of the tumor with nuclear polymorphism, mitoses, and gland formation (original magnification ×200). c Hematoxylin-eosin of lymph node tissue with nearly complete replacement of the normal lymph node with tumor (original magnification ×20).
**Fig. 2.** Lymph node tumor stained with either a pan-p53 monoclonal antibody (original magnification ×200), b p63 monoclonal antibody (original magnification ×200), c p73 monoclonal antibody (original magnification ×200), or d ΔNp63 monoclonal antibody (original magnification ×200).
Fig. 3. Schematic of the TA and ΔNp63 exon structures and gene products as predicted in the 'two genes in one'-model. TA = Transactivation domain; ΔN = delta N domain; PR = proline-rich domain; DNB = DNA-binding domain; OD = oligomerization domain; SAM = sterile α-motif.
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