Differentiated-Type Intraepithelial Neoplasia-Like Lesion Associated with Squamous Cell Carcinoma of the Anus: A Case Report with Molecular Profile

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Differentiated-type Intraepithelial Neoplasia (DIN) is defined as HPV-negative squamous intraepithelial proliferation with abnormal keratinocyte differentiation and basal cell atypia [1]. This pathological entity was originally described in the vulva, with following descriptions in the oral cavity [2–5] and the genitourinary tract, especially the penis [6–8]. In the vulva, this lesion is associated with lichen sclerosus or planus and often associated with keratinizing squamous cell carcinoma (SCC).

To the best of our knowledge, only one publication reported DIN in the anus [9]. Terminology concerning this lesion is confusing as it is not described in the WHO Classification of Tumours of the Digestive System. Indeed, precursor lesions reported in the anal canal are “classical” anal intraepithelial neoplasia, mostly HPV-related [10]. The prevalence of HPV infection in anal carcinoma (84.3%) approaches that reported in cervical carcinoma (87.3%), for which HPV infection is considered a necessary cause. HPV prevalence is much lower in vulvar carcinoma (40.4%) [11].

In the vulva, DIN is classically associated with TP53 mutations and will be p53 immunopositive when missense mutations are present. Some cases shared identical TP53 mutations in both DIN and SCC [12].

Therefore, the aim of the present study is to assess the molecular profile of this entity in the anus using the next generation sequencing (NGS) technique in correlation with immunohistochemical data.

1. Introduction

Differentiated-type Intraepithelial Neoplasia (DIN) is defined as HPV-negative squamous intraepithelial proliferation with abnormal keratinocyte differentiation and basal cell atypia [1]. This pathological entity was originally described in the vulva, with following descriptions in the oral cavity [2–5] and the genitourinary tract, especially the penis [6–8]. In the vulva, this lesion is associated with lichen sclerosus or planus and often associated with keratinizing squamous cell carcinoma (SCC).

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2. Case Presentation

A 59-year-old man presented in December 2017 an indurated lesion of the anal margin causing burning sensation, measuring 1 cm (Figure 1).
The biopsy revealed moderately differentiated squamous cell carcinoma. Using immunohistochemistry, irregular/heterogenous positivity for p16 protein was observed (Figure 2).

The detection of High Risk-HPV DNA (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 66, and 68) from the paraffin-embedded sample using the BD onclarity HPV assay (BD diagnostics, Sparks, USA) was negative [13].

The tumor was classified cT1 and treated by radiotherapy until February 2018. In May 2018, after a period of complete response, the patient noted the reappearance of an indurated and painful area near the anal margin. The patient underwent excision in June 2018.

Macroscopically, an irregular and ulcerated lesion occupying the near totality of a mucous ellipse measuring 26x15 mm was observed. This lesion was covered by a white coat.

Microscopically, the tumor consisted of nests of invasive squamous cell carcinoma, moderately differentiated. Lateral margins were positive. Using immunohistochemistry, tumor was negative for p16 (clone ink4a E6H4, ready to use, Roche). Immunoreactivity of p53 (clone DO-7, 1:200, Dako Agilent) appeared continuous and limited to the periphery of invasive nests, with strong intensity (Figure 3). The tumor was classified rpT2Nx.

Gene mutation testing was performed by NGS, as we have previously described [14, 15], with a panel of 50 genes described in Table 1. One mutation was found: G279fs∗4 (c.833.834insGAGTCGAAACTCCACGCACAAACACGACAGGAC) frameshift mutation of the TP53 gene.

In addition, the detection of High Risk-HPV DNA was negative[13].

According to all these pathological data, the diagnosis of differentiated-type intraepithelial neoplasia (DIN) was suggested.

Gene mutation testing was performed in this DIN-like lesion, but no mutation was found.

### Table 1: Cancer panel used by NGS.

| Gene | Gene | Gene | Gene | Gene |
|------|------|------|------|------|
| ABL1 | EGFR | GNAQ | KRAS | PTPN11 |
| AKT1 | ERBB2 | GNAS | MET | R1B |
| ALK | ERBB4 | HNFA | MLH1 | RET |
| APC | EZH2 | HRAS | MPL | SMAD4 |
| ATM | FBXW7 | IDH1 | NMP1 | SMARC1 |
| BRAF | FGFR1 | IDH2 | NOTCH1 | SMO |
| CDH1 | FGFR2 | JAK2 | NRAS | SRC |
| CDKN2A | FGFR3 | JAK3 | PDGFR| STK11 |
| CSF1R | FLT3 | KDR | PIK3CA | TP53 |
| CTNNB1 | GNAI1 | KIT | PTEN | VHL |

DIN is a subtle and difficult histopathological diagnosis, with a low interobserver agreement [21].

Histological and immunohistochemical characteristics present overlap with other entities, such as lichen sclerosus, squamous cell hyperplasia, or inflammatory disorders. Increased p53 staining can be seen in 5-61% of lichen sclerosus and up to 40% of squamous cell hyperplasia and
Figure 2: Microscopic aspect on biopsy, revealing moderately differentiated squamous cell carcinoma (a), with irregular/heterogenous positivity for p16 immunohistochemistry (b).

Figure 3: Microscopic and immunohistochemical aspects of 1st excision. Tumor consisted of nests of invasive squamous cell carcinoma, with focal keratinization (a). p53 appeared continuous and limited to the periphery of invasive nests, with strong intensity (b).

Figure 4: Continued.
is thought to be due to increased oxidative stress. Moreover, some authors suspect that atypical lichen sclerosus, showing increased p53 staining, may represent a very early form of DIN [22].

Therefore, we believe these entities are a spectrum of lesions sharing common histological features, where TP53 mutation could be a further event in anal SCC carcinogenesis.

In conclusion, we described a potential precursor lesion of SCC in the anus analogous to DIN in the oral cavity and vulva. The recognition of such a precursor should lead to a careful analysis of the HPV status and the molecular profile of cancer to detect the presence of TP53 mutations. Furthermore, studies investigating prognostic impact of such mutations in DIN-like lesions and associated SCC in the anus are warranted.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest regarding the publication of this article.

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