protein (pRB)-phosphorylation. This indirectly could represent an aberrant function of pRB pathway, suggesting a different pathogenic mechanism involved in FH mutated cancers. Regarding alpha-SMA, recent data suggest that overexpression of Retinoblastoma-binding protein 2 (RBP2) can increase alpha-SMA levels; conversely, the gene knockdown of RBP2 expression decreases levels of alpha-SMA. RBP2 is a nuclear histone demethylase implicated in epigenetic transcription regulation of a wide range of genes through the pRB and p16 tumour suppressor proteins. An aberrant function of pRB pathway could lead to down-regulation of RBP2; this, in turn, would decrease the HIF expression levels, with a consequent reduction in alpha-SMA expression.

We report for the first time a detailed p16, FH, and alpha-SMA protein expression study of cutaneous leiomyoma in FH genotyped patients. We observed that p16, FH, and alpha-SMA expression patterns appear to be correlated with the genotypic status of the FH gene. Our IHC FH findings are in agreement with what has already been described in the literature in patients affected by sporadic PLM (positive expression) and HLRCC-PLM (negative or weak positive). Regarding alpha-SMA expression, we observed a negative IHC and IF expression in sporadic PLM, unlike HLRCC-PLMs that showed an intense reactivity for this protein.

Our data could suggest a possible new aetopathogenetic hypothesis for NH-CL. These findings would support a different tumourigenic mechanism of CL in wild-type FH patients, compared to FH germline mutation carriers. Since PLM of wild-type FH subjects showed FH and p16 over-expression, and concomitant negative expression of alpha-SMA, the potential pathogenic role of an aberrant (inactive, non-functional) Rb-pathway could be hypothesised, similar to that reported in the literature for other skin and non-skin non-functional) Rb-pathway could be hypothesised, similar to that reported in the literature for other skin and non-skin cancerous conditions.

Here we also describe for the first time clinical, IHC, IF and genetic features of a diffuse MCL in a wild-type FH patient (NH-MCL, Patient 1).

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APPENDIX A. SUPPLEMENTARY DATA
Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.05.006.

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Molecular analysis of melanocytic naevus arising from ovarian mature teratoma

Sir,

Melanocytes are cutaneous or extracutaneous, such as uveal melanocytes, and they are all considered of neural crest origin except for the retinal pigmented epithelial cells, which originate from neural ectoderm of the developing forebrain. Melanocytic naevi are clonal proliferations of melanocytes comprising different subtypes such as congenital, acquired, blue and Spitz naevi, each with a different genetic background and driver mutation: acquired naevi harbour BRAF mutations; congenital naevi NRAS and, to a lesser extent, BRAF mutations; blue naevi GNAQ mutations; and Spitz naevi HRAS mutations. In melanomas, BRAF mutations are more common in tumours not arising in the context of chronic sun damage. Melanocytic tumours usually involve the skin, while extracutaneous sites are rarer. An extremely rare phenomenon is the development of melanocytic tumours inside...
an ovarian teratoma with only a very few cases described in the literature. A 24-year-old female was incidentally diagnosed with a left ovary cystic lesion with imaging findings of cystic teratoma (dermoid cyst). The cyst was excised laparoscopically and sent for histopathological evaluation which showed a unilocular 2.7 cm cyst filled with sebaceous material and hair. Microscopic examination confirmed the diagnosis of ovarian cystic teratoma showing a predominance of skin tissues and a few mesodermal derivatives (Fig. 1). Furthermore, inside the skin tissue area, a 3 mm melanocytic proliferation with features of compound naevus was found. Naevocytes were arranged in nests showing maturation (Fig. 1 and 2); the lesion showed some architectural irregularity, but the epidermis of the teratoma was also irregular in comparison to normal skin. No significant atypia, mitotic activity or necrosis were seen. Lesional melanocytes expressed S100 and Melan A, without HBM45 expression. P16 expression was retained, while MiB1 was less than 1%. For molecular analysis, tumour DNA was extracted from 8-μm thick sections of formalin-fixed, paraffin-embedded (FFPE) tissue after dewaxing. Automated DNA extraction was performed on Qiacube using DNA FFPE kits (Qiagen, France). DNA concentration was measured using the Qubit dsDNA HS assay kit (ThermoFisher Scientific, USA). For library preparation, 10 ng of DNA was amplified using the AmpliSeq CE-IVD Colon and Lung Cancer Panel kit (ThermoFisher Scientific). This generated 192 amplicons and 22 genes were analysed: \( \text{AKT1} \) (NM_05163), \( \text{ALK} \) (NM_004304), \( \text{BRAF} \) (NM_004333), \( \text{CTNNB1} \) (NM_001904), \( \text{DOR2} \) (NM_001014796), \( \text{EGFR} \) (NM_005228), \( \text{ERBB2} \) (NM_004448), \( \text{ERBB4} \) (NM_005235), \( \text{FBXW7} \) (NM_033632), \( \text{FGFR1} \) (NM_023110), \( \text{FGFR2} \) (NM_022970), \( \text{FGFR3} \) (NM_000142), \( \text{KRAS} \) (NM_033360), \( \text{MAP2K1} \) (NM_002755), \( \text{MET} \) (NM_001127500), \( \text{NOTCH1} \) (NM_017617), \( \text{NRAS} \) (NM_002524), \( \text{PIK3CA} \) (NM_006218), \( \text{PTEN} \) (NM_000314), \( \text{SMAD4} \) (NM_005359), \( \text{STK11} \) (NM_000455), \( \text{TP53} \) (NM_000546). Library multiplexing, clonal amplification on Ion Sphere particles (ISP) by emulsion polymerase chain reaction (PCR) and loading on 318 chip were performed on the Ion Chef instrument with Hi-Qview sequencing kit (Thermofisher Scientific). Finally, the template ISP sequencing was performed on an Ion PGM with 200 kit v2 according to the manufacturer’s instructions. Next generation sequencing (NGS) data analysis was performed with Ion Reporter 5.6 Software and Alamut (Interactive Biosoftware, France). \( \text{BRAF} \) V600E was the only mutation found. Confirmation of \( \text{BRAF} \) mutation was carried out with SNaPshot (Applied Biosystems, USA). PCR assay was designed to amplify fragments of \( \text{BRAF} \) exon 15 (224 pb). PCR was performed according to the Qiagen Hot Star protocol (Qiagen, Germany) in a total volume of 50 μL. The purified PCR were labelled using a SNaPshot Multiplex Kit (Applied Biosystems). SNaPshot products were then purified with shrimp alkaline phosphatase and 2 μL of the labelled products were analysed on the ABI PRISM 3130 DNA analyser (Applied Biosystems) with GeneMapper software (ThermoFisher Scientific).

Ovarian mature cystic teratomas are one of the most frequent ovarian tumours comprising almost 20% of all ovarian neoplasms and occurring most usually during the reproductive age. They belong to the germ cell tumours of the ovary and the principal theory of their origin is parthenogenesis, suggesting an origin from the primordial germ cell, having a 46,XX karyotype. They are composed of various tissue types representing two or three germ cell layers: ectoderm, mesoderm, endoderm. They are benign tumours; the malignant counterpart, called immature teratoma, contains immature tissues and is less frequent. Malignant transformation of an otherwise mature teratoma under the form of somatic type malignancy is rare and usually presents as squamous cell carcinoma, probably due to the abundance of skin tissues in this tumour. Despite this abundance of skin tissues in mature teratomas, the frequent presence of epidermal melanocytes in these

![Fig. 1](image-url) (A) The ovarian cyst composed mainly of skin tissues [hematoxylin, eosin, safran (HES)]. Inside the marked area, a pigmented lesion. (B) At higher magnification naevocytes show nested pattern in the dermis (HES). (C) Junctional component (HES). (D) Protein S100 expression from melanocytes (3,3'-diaminobenzidine).
tumours and even in teratomas experimentally formed after transplantation of undifferentiated embryonal stem cells in subcutaneous tissues, melanocytic lesions in mature teratomas are extremely rare. This could represent the actual rarity of the lesion or it could be due to underdiagnosis, inadequate sampling or not reporting of these lesions. Rare cases of dermal, compound or dysplastic naevi, as well as melanomas, have been reported, representing a rare phenomenon of a ‘neoplasm inside a neoplasm’. These naevi were reported with teratomas that were large (range 4 cm to 10.5 cm), while in our case a small 3 mm naevus inside a small teratoma was found. Molecular characterisation of these melanocytic lesions has never been performed. We show here that melanocytic naevus arising inside mature teratoma harbours \textit{BRAF} mutation, the most common alteration of acquired naevi.

Thus, melanocytes existing inside the skin tissues of ovarian teratomas, away from sun exposure, can undergo the same mutations and give the same clonal proliferations as melanocytes of normal epidermis.

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\textbf{Myoepithelioma-like tumour of the vulvar region}

\textit{Sir.}

Myoepithelioma-like tumour of the vulvar region (MELTVR) is a rare mesenchymal neoplasm of the vulvar area. Histologically, MELTVRs are usually similar to soft

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{(A) The lesion shows maturation, as expected in classical skin naevi [hematoxylin, eosin, safran (HES)]. (B) P16 expression is retained [3,3'-diaminobenzidine (DAB)]. (C) No invasion of the overlying epidermis is seen (HES). (D) MiB1 expression is seen in a very few naevocytes (DAB).}
\end{figure}