CORRESPONDENCE

Stemness in high-grade serous carcinoma of tubo-ovarian origin causes multiple immunohistochemical pitfalls: a case report

High-grade serous carcinoma (HGSC) of tubo-ovarian origin is a malignant neoplasm with heterogeneous morphology. This intratumour morphological heterogeneity comprises various architectural patterns and diverse cytological aspects, which often coexist within a single tumour.[1] Although HGSC is characterised by a high mitotic rate and substantial nuclear pleomorphism, architecturally and/or cytologically diverse areas can cause diagnostic confusion.[1] The current case illustrates that clear cell changes in HGSC can provoke 'blunderbuss immunohistochemistry',[2] leading the pathologist astray and resulting into academic referral because of unexpected staining patterns.

A woman in her 70s underwent a bilateral salpingo-oophorectomy because of an enlarged right ovary. This ovary contained a predominantly solid and partially cystic mass, measuring 4.5×3×2.5 cm. The left ovary and both Fallopian tubes did not show any anomalies. Peritoneal carcinomatosis was not observed. Histopathological evaluation of the left ovary and both Fallopian tubes was normal. Sampling of the unilateral right ovarian mass revealed a neoplasm with prominent cytonuclear atypia, numerous mitoses (including several atypical mitotic figures) and extensive necrosis. The tumour had a mixed solid, cribriform and glandular architecture. Because of some solid clear cell areas, immunohistochemical stainings for CD117 and placental alkaline phosphatase (PLAP) had initially been performed to exclude the possibility of a dysgerminoma component. The tumour showed limited areas with moderate PLAP expression and weak CD117 staining (figure 1). There was focal weak immunoreactivity for cytokeratin-7 and p16. This case was subsequently referred to an academic laboratory for further investigation.

Additional immunohistochemistry showed focal strong staining for glypican-3 and podoplanin (D2-40) and diffuse nuclear staining for paired box protein 8 (PAX8) and Wilms tumour 1 (WT1). The tumour showed no immunoreactivity for nuclear octamer-binding transcription factor 4 (OCT4), focal weak expression of Sal-like protein 4 (SALL4) and intense membranous staining for epithelial membrane antigen (EMA) and CK-AE1/AE3 (figure 2). The tumour nuclei showed a total lack of p53 immunoreactivity, with weak-to-moderate nuclear expression in fibroblasts and lymphocytes, which corresponded with an aberrant mutation-type p53 staining pattern.[3] Targeted next-generation sequencing showed three different *TP53* gene alterations (c.421_424del, c.428T>G and C.433_435del), all with a variant allele frequency of 61%–63% and all considered (probably) pathogenic by COSMIC (https://cancer.sanger.ac.uk/cosmic) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The immunohistochemical profile corresponded with an HGSC, since its epithelial origin was indicated by strong immunoreactivity for EMA and CK-AE1/AE3.[4] Contrariwise, dysgerminomas are EMA negative and usually present focal dot-like CK-AE1/AE3 staining.[4] The absence of diffuse 'block-type' p16 immunoreactivity does not exclude an HGSC, since around one-third of HGSCs shows only focal p16 expression.[4] Dysgerminomas generally express CD117, PLAP and podoplanin,

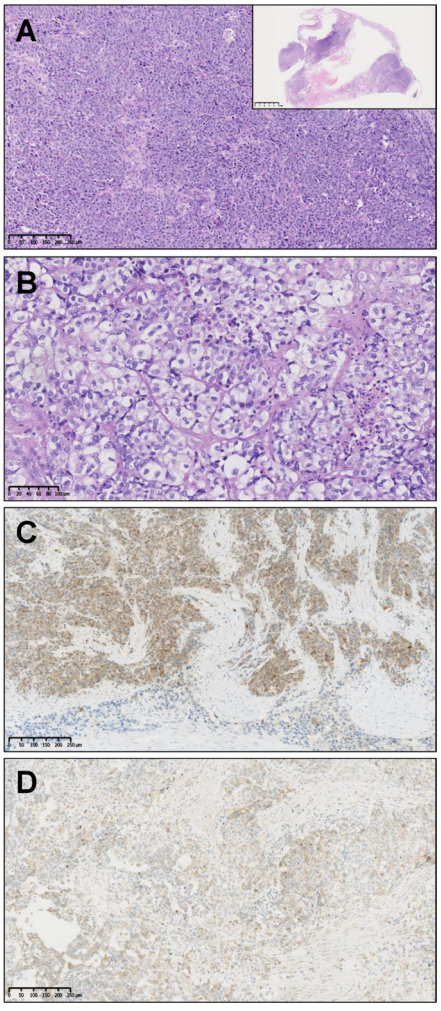

Figure 1 H&E staining showed large solid areas with important nuclear pleomorphism (A; original magnification 100×) in a partially cystic tumour (inset A), as well as some clear cell areas (B; original magnification 200×). Immunohistochemistry revealed focal placental alkaline phosphatase expression (C) and weak staining for CD117 (D; original magnification 100×).

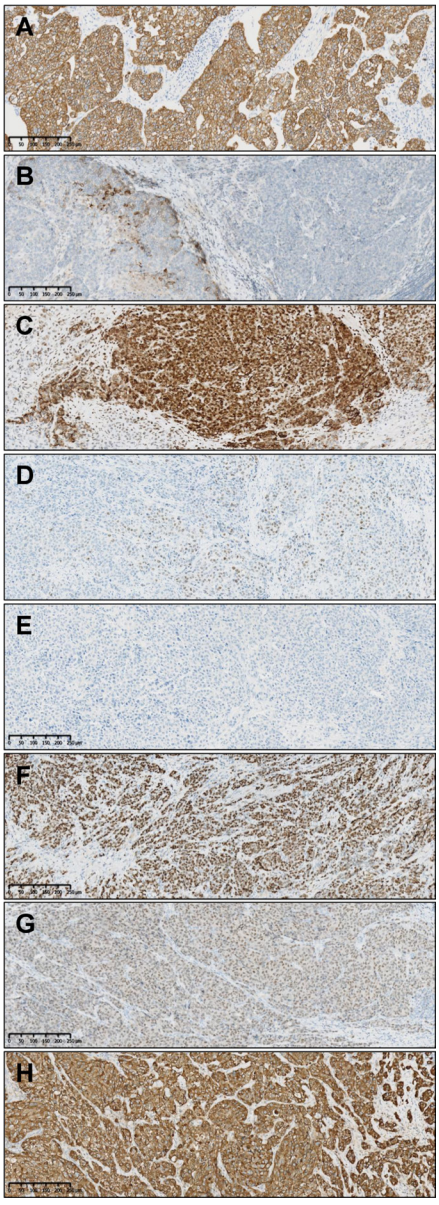

Figure 2 Immunohistochemistry (original magnification 100×) for broad spectrum cytokeratin (A) AE1/AE3, (B) glypican-3, (C) podoplanin, (D) Sal-like protein 4, (E) octamer-binding transcription factor 4, (F) Wilms tumour 1 (WT1), (G) paired box protein 8 and (H) epithelial membrane antigen.

and occasionally also express glypican-3.[4] However, these markers are not specific for dysgerminomas and their expression has been previously described in HGSC.[4–6] Similarly, up to 20% of HGSCs express SALL4 to some extent,[7] as was the case in the lesion we present here. Although no marker is 100% sensitive or specific for anything, the pluripotency marker OCT4 was useful here to confirm the diagnosis of HGSC. Focal OCT4 immunoreactivity in HGSCs has been reported, but dysgerminomas are nearly always diffusely positive.[4] Almost all tubo-ovarian HGSCs present with WT1 immunoreactivity, and >95% of HGSCs harbour a *TP53* mutation with associated 'mutation-type' p53 staining.[3–5] *TP53* mutations in dysgerminomas have not yet been described.[8] However, 44% of dysgerminomas present with pathogenic *KIT* mutations and 82% harbour a chromosome p12 gain,[8] allowing to further differentiate dysgerminomas from HGSCs at the molecular level.

We surmise that the focal immunoreactivity for podoplanin, PLAP, glypican-3 and CD117 in this HGSC is caused by dedifferentiation or acquisition of the so-called 'stemness' by the tumour cells. Stemness reflects the acquisition of pluripotency, probably indicating a more aggressive behaviour. Little is known about the role of stemness in the maintenance of tumour progression or the development of therapeutic resistance. HGSCs are generally diagnosed as late-stage disease and therefore generally have a poor prognosis. The acquisition of stemness might contribute to an even worse overall survival. It might be interesting to explore the expression of markers of pluripotency as prognosticators in HGSC, especially in early-stage disease.

In conclusion, this HGSC case illustrates that clear cells can occur in HGSC, and that acquisition of stemness can pose a substantial diagnostic challenge when interpreting immunohistochemistry. Pathologists should bear in mind that no immunohistochemical staining is 100% sensitive or specific for anything. Before ordering immunohistochemistry, the pathologist should answer the questions: 'which pattern of immunoreactivity do I expect, and will this positive/negative/normal/aberrant result help me to establish the diagnosis?'.[2] If the answer is unknown, it seems better to not ask for that particular staining. In general, a panel of WT1, PAX8 and p53 is sufficient to confirm a morphological suspicion of tubo-ovarian HGSC. If necessary, this basic panel can be supplemented with deliberated additional stainings and molecular analysis to differentiate HGSC from other tumour types.

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