LETTER TO THE EDITOR

Caution over use of ES2 as a model of ovarian clear cell carcinoma

Dear Sir,

Clear cell carcinoma (CCC) of the ovary is a subtype of epithelial ovarian cancer (EOC) known for its relative resistance to standard platinum-based chemotherapy and poor prognosis. CCC account for up to 25% of ovarian carcinomas in Japan while make up only about 10% in North America. Analysis of archived pathology records on cases of ovarian cancer managed by Queen Mary Hospital, Hong Kong, from January 1993 to June 2012 revealed that CCC constituted 22.4% of EOC locally. Moreover, its yearly incidence showed a significant increase (unpublished results). Studies on ovarian CCC are therefore important and of regional significance.

Genetic and molecular biological studies of ovarian CCC often involve in vitro experiments using several widely recognised ovarian CCC cell lines including ES2, TOV-21G, OVISE, OVTOKO and OVMANA. However, we found that ES2, a widely used ovarian CCC cell line established in the late 1980s, displayed unusual features distinct from other ovarian CCC cell lines. Such observations led us to question its true histological identity.

Verification of hepatocyte nuclear factor 1β (HNF-1β) expression in our ovarian cancer cell lines has been carried out. HNF-1β is a transcription factor found to be considerably upregulated in almost all ovarian CCC through CpG island hypermethylation but not in other histological subtypes of ovarian cancers. It is therefore regarded as a histotype-specific biomarker. As we checked for the expression of HNF-1β in our cell lines using immunocytochemistry, strong nuclear staining was observed in the ovarian CCC cell lines OVMANA, OVISE and OVSAYO, while staining in the serous carcinoma (SC) cell line OVCAR-3 was negative as expected. In contrast, no such nuclear expression of HNF-1β was found in ES2 cells (figure 1A).

Figure 1 ES2 displays an immunohistochemical staining pattern atypical to ovarian clear cell carcinoma. (A) Ovarian cancer cell lines were stained for hepatocyte nuclear factor 1β (HNF-1β) or Wilm’s tumour 1 (WT1) which are immunohistochemical markers for ovarian clear cell carcinoma and high grade serous carcinoma, respectively. (B) Western blotting analysis of HNF-1β in the cell lines.
Such unexpected findings concurred with western blotting results that revealed the lack of HNF-1β expression in ES2 (figure 1B). Short tandem repeat (STR) profiling analysis showed that the STR profiles of our cell lines were consistent with reference profiles from both the American Type Culture Collection and the Japanese Collection of Research Bioresources databases, verifying the authenticity of all the cell lines used.

Further literature review revealed several genetic features of ES2 that suggested its atypicality as an ovarian CCC cell line. TP53 mutations are present in 97% of high grade SC (HGSC), most common of which were missense mutations causing nuclear accumulation of the mutant protein. In contrast, TP53 mutations in ovarian CCC are rare, whereas ARID1A and PIK3CA mutations are common in ovarian CCC. Such genetic disparity is considered to be one of the important features in differentiating between type I and II EOCs, to which CCC and HGSC belong, respectively. Tan et al characterized the p53 statuses of 12 CCC cell lines (including ES2, OVMANA, OVISE and OVSAO used) by sequencing known mutational hotspots, and the results demonstrated that ES2 was the only CCC cell line harbouring TP53 mutation (c. 722C>T, p.S241F). Consistent with its suggested HGSC origin, OVCAR-3 has mutated TP53. In addition, genomic profiling demonstrated the absence of ARID1A and PIK3CA mutations in OVCAR-3 as well as ES2 whereas the two genes are mutated and inactivated in both OVMANA and OVISE. Therefore, the genetic profile of ES2 is more closely related to HGSC than to CCC.

We further validated the true histological typing of ES2 by performing immunohistochemical staining on Wilms’ tumour 1 (WT1) which exhibits high specificity (97.4%) for SC. WT1 gene encodes a zinc finger transcription factor which regulates growth factor genes and is frequently expressed in SC but is commonly repressed in CCC owing to promoter methylation. Our immunohistochemical results confirmed the expression of WT1 in the SC cell lines OVCAR-3, and its lack of expression in CCC cell lines OVMANA, OVISE and OVSAO. The strong cytoplasmic and nuclear staining for WT1 in ES2 further confirmed a SC-like immunophenotype (figure 1A).

From our findings, we conclude that ES2 exhibits an immunohistochemical pattern compatible with that of HGSC with HNF-1β negativity and WT1 positivity. This profile is the exact opposite to that of conventional ovarian CCC.

ES2 was originally described as a cell line established from a poorly differentiated CCC, derived from the surgical tumour specimen of a 47-year-old black woman. A high-grade cytology might sometimes present difficulties when differentiating between CCC and SC. In fact, the distinction between CCC and SC has been recognised as a potential diagnostic challenge, owing largely to the morphological diversity of CCC and SC. In addition, the presence of more than one cell type within the same tumour could be another factor contributing to diagnostic uncertainties. Mixed EOCs with both clear cell and serous components have been demonstrated to display clinical and immunophenotypical characteristics indistinguishable from those of HGSCs. Specifically, the ‘clear cell components’ within the mixed EOCs were found to possess relatively high mitotic activities, WT1 positivity and p53 overexpression as opposed to their serous counterparts in tumours and also in pure HGSC. Thus, the presence of serous or other EOC components must be carefully ruled out before the diagnosis of pure CCC is reached.

In summary, we propose that ES2 should more appropriately be regarded as an SC rather than a CCC cell line in light of our findings. We thereby suggest that this cell line should be used with caution in studies characterising the behaviour of CCC or type I EOC to avoid reaching unreliable results leading to doubtful conclusions.

**REFERENCES**

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J Clin Pathol  published online July 21, 2014

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