In vitro transformation of ovarian surface epithelium links a mutation in SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 4 to familial small carcinoma of the ovary hypercalcemic type

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Topic: Ovarian

Objectives
Using a unique population of cells derived from a familial carrier of an SCCOHT-associated SMARCA4 mutation (SMARCA4. c.3081+1G>T), we sought to determine if non-cancerous cells would spontaneously transform during in vitro culture, thereby providing a model system for studying the formation of SCCOHT.

Methods
Ovarian surface epithelium (OSE) derived from a familial carrier of a SCCOHT-associated mutation in the SMARCA4 gene (P590, SMARCA4. c.3081+1G>T, prophylactic oophorectomy) were serially passaged in vitro, and interrogated for their ability to acquire characteristics of cellular transformation. Cells were grown for multiple passages to determine if cells would spontaneously immortalize. Loss of contact-dependent inhibition was interrogated with focus-forming assays of confluent monolayers. The ability of cells to escape anoikis and grow independently as spheroids was interrogated in attachment-free growth conditions. Senescence-associated beta-galactosidase (SA-beta-gal), cellular necrosis, and cellular apoptosis was also measured. All experiments were conducted in parallel with an age-matched OSE population derived from a benign ovarian surgery.

Results
P590 cells that were passaged over 12 twelve times, representing at least 40 cellular doublings, continued to proliferate in culture. In contrast, non-mutant P583 cells failed to grow beyond 11-12 passages. P590 cells grown to confluency spontaneously formed foci of multicellular aggregates, while P583 cells did not. In attachment-free culture conditions, P590 cells readily formed spheroids from both early-passage and late-passage cells. In contrast, P583 formed small spheroids that failed to expand in culture. Surprisingly, both P590 and P583 cells displayed elevated SA-beta-gal staining, with neither cell populations showing elevated necrosis or apoptosis markers.

Conclusions
OSE harboring a SMARCA4 mutation associated with familial SCCOHT display several phenotypes consistent with early stages of cellular transformation. In contrast, non-mutant cells failed to display signs of transformation. Our data suggest that OSE may represent a unique cell population for studying the early stages of SCCOHT formation, providing a useful tool for developing improved diagnostic and therapeutic approaches.