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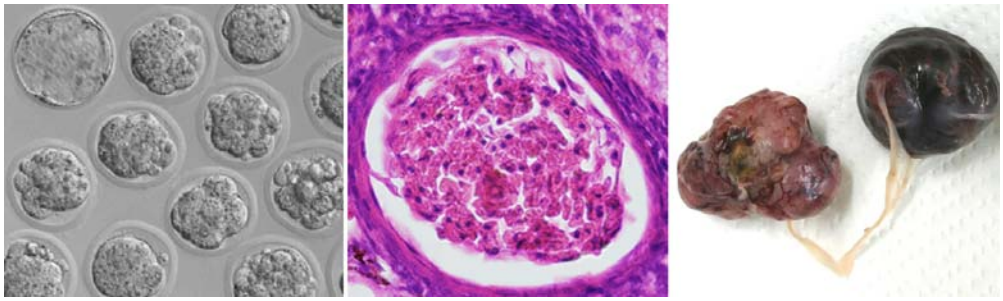
Link between fertilization and tumor formation in phospholipase C zeta

November 1, 2007 – Mammalian oocytes arrest at a point of the cell cycle called metaphase II (mII). The purpose of this arrest is to prevent the initiation of embryonic development until the arrival of a fertilizing sperm, which sends a signal to the oocyte to stop behaving like an egg and start behaving like an embryo. This transformation includes release from mII arrest, resumption of the cell cycle and the initiation of the developmental program. But the nature of the sperm-borne signal and the means by which it triggers fertilization remain have proven to be elusive quarry. The Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) previously showed that one component of the sperm signal corresponds to the phospholipase C (PLC) isoform PLC-zeta, PLCZ1. This jibed nicely with the view that sperm prompt meiotic resumption by generating waves of calcium ions, because PLCs in general are able to induce ionic calcium release by hydrolyzing membrane phospholipids. One problem with this idea is that oocytes have PLCs of their own, albeit different isoforms than PLCZ1, begging the question of why PLCZ1 should specifically have the signaling effect it is proposed to have.



Three stages of tumour formation. Oocytes (eggs) from transgenic, PLCZ1-expressing mice initiate development by themselves (parthenogenesis, left panel), which may seed early ovarian tumors (center) that grow into large masses.

Now, in report published in the journal *Development*, Naoko Yoshida and colleagues have extended their studies to address these questions. In particular, they wanted to get a better idea of whether PLCZ1 is able to induce cell cycle progression in oocytes in the absence of other sperm proteins, and if so, whether this activity really was specific to oocytes. Using a transgenic approach, they first determined that *Plcz1* mRNA transcripts are found in the brains of both sexes and in the testis, allowing them to devise experiments in which its expression could be driven ectopically, in sites from which the mRNAs (and presumably protein) are typically absent, including oocytes. To do this, the team focused on two mouse lines with different transgenes in which *Plcz1* expression was driven by a broadly active promoter.

The transgenic lines behaved similarly to each other. Pups were born and developed normally at first. However, females exhibited very low fertility. Their oocytes underwent normal maturation, arriving at mII just like those of their non-transgenic littermates. But unlike normal oocytes, the ones from transgenic mice then immediately progressed to anaphase II and beyond, as if they had been fertilized, often developing to the blastocyst stage in vitro. These findings indicate the exquisite specificity of PLCZ1, which enables it to induce parthenogenetic exit from meiosis II (it is even specific to a particular stage of a particular cell-cycle) in otherwise healthy mice. “This is the first demonstration of ovarian teratoma formation in mice whose oocytes complete meiotic maturation,” according to Perry.

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The work doesn't categorically address whether additional sperm proteins are normally required to lower the threshold of the PLCZ1 signal, because the amount of transgenic PLCZ1 in the oocytes was too small to measure. But the group were able to show that the PLCZ1 does act directly. When they transferred *Plcz1* transgenic cumulus cell nuclei into wild-type oocytes, the oocytes frequently became activated and could develop to the blastocyst stage. It remains to be seen whether the PLCZ1-expressing cells of these mice support full development as if they were 'somatic sperm'.

Although *Plcz1* transgenic mice initially appeared normal and healthy, females developed abdominal swellings caused by ovarian tumors. The frequency of tumour development in females was high (~70% after 6 months) but tumors were never found in transgenic males, corroborating the specificity of the phenotype. The simplest explanation for the tumors is that PLCZ1-induced parthenogenesis occasionally occurred in mature oocytes that failed to be ovulated, and that the resultant trapped parthenogenotes subsequently underwent unchecked ovarian growth to yield the tumors. Questions, however, remain. Most tumors, although occurring in a hemizygous background, are apparently hemizygous, yet the 'tumor-from-parthenogenote' model doesn't readily explain how. In addition, the parthenogenotes often underwent uterine implantation, but never induced uterine tumorigenesis. It is unclear why ovarian, but not uterine, sites should foster tumour formation. The tumors in some cases accounted for a large proportion of the total body mass, so given their embryonic etiology it is unclear why metastasis was never observed.

Several of the PLCZ1-expressing transgenic mice developed ataxia, a presentation for a subset of clinical ovarian cancer patients. However, the group did not find evidence for mutations in the *PLCZ1* gene in human breast epithelial, ovarian epithelial or benign ovarian germline tumors. Nevertheless, PLCZ1-expressing transgenic mice provide a tractable model for the study of ovarian tumor development, and indicate that PLCZ1 provides an intriguing link between fertilization and tumorigenesis.

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