Preserving the oocyte reserve

By Tracey Baas, Senior Editor

A **Cornell University** team has shown how knocking out *checkpoint kinase 2* can preserve fertility in mice.[1] The results suggest that companies have an opportunity with this kinase, which is less pursued than the well-trodden cancer target checkpoint kinase 1, to prevent chemotherapy- and radiotherapy-induced premature menopause and infertility.

Oocytes that contain genetic errors, such as unrepaired DNA double-strand breaks (DSBs), can lead to birth defects and spontaneous abortions. Under normal conditions, the body repairs such breaks by homologous recombination.

Known molecular repair mechanisms in humans include ataxia telangiectasia mutated (ATM) kinase, which responds primarily to DSBs, and ataxia telangiectasia and Rad3 related (ATR; FRP1), which responds primarily to single-stranded DNA breaks.[2,3]

If these repair mechanisms fail to correct the mutations, unknown checkpoint triggers are activated that lead to elimination of defective oocytes and possible primordial ovarian follicle depletion. The result can be infertility.

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—Antonio Giordano, Temple University

In women undergoing cancer radiotherapy or chemotherapy, the incidence of genetic damage is substantially elevated, often leading to oocyte and ovarian follicle depletion and to premature ovarian failure and menopause.

In these patients, options to preserve fertility are oocyte or ovarian tissue cryopreservation or gonadal suppression with gonadotropin-releasing hormone (GnRH) agonist therapy. The former approach works but does not impede premature menopause. The latter approach has yielded mixed results and is not an option for women with estrogen-dependent tumors.

Previous research has shown that Atm and Atr are needed to ensure fertility and oocyte viability in mice.[2] However, a downstream effector of Atm kinase called checkpoint kinase 2 (Chk2; Chek2) was not required.

Those findings prompted a team led by John Schimenti to hypothesize that inhibiting this checkpoint effector could reduce the number of oocytes that are eliminated while allowing other mechanisms to complete DSB repair and maintain viable oocytes.

Schimenti is a professor of genetics and director of the Center for Vertebrate Genomics at Cornell.

In female mice with genetically induced meiotic failure, Schimenti's team showed that *Chk2* deficiency increased the number of ovarian follicles compared with normal *Chk2* expression. Oocytes in *Chk2*-deficient mice were viable, despite abundant DSBs, and resulted in multiple litters of pups with no visible abnormalities at one year of age.

Similar results occurred in mice with DSBs induced by irradiation. Irradiated wild-type animals showed complete elimination of the follicle pool, whereas *Chk2*$^{-/-}$ mice retained follicles, did not undergo DSB-mediated oocyte elimination and remained fertile.

The knockouts produced litter sizes that were comparable to those of unirradiated controls, and the resulting pups did not exhibit visible abnormalities at one year of age.

Results were published in *Science*.

Deeper dive

The team's next steps include sequencing the genomes of the pups to check for potential mutations and other defects and identifying the mechanism responsible for repairing oocyte DSB damage.

"Because females with DSBs were able to produce litters of pups that showed no visible abnormalities, the results suggest that all or most DSBs were eventually repaired," said Schimenti, who is corresponding author on the paper. "We have genetic experiments under way to identify the mechanism responsible for repairing the remaining DNA damage and also to determine what other types of defects are monitored by CHK2. We will also be sequencing the genomes of the mouse pups to check for mutations."

Schimenti also said that the team is testing CHK2 inhibitors in mouse ovaries. "We are almost certain that the checkpoint pathways work the same in mice and humans; the basic pathway even exists in yeast," he said.

AstraZeneca plc's AZD7762, a CHK1 and CHK2 inhibitor, was in Phase I testing for cancer as monotherapy and in combination with gemcitabine or irinotecan but has now been terminated.

A more selective CHK2 inhibitor, CCT241533 from **The Institute of Cancer Research**'s **Cancer Research UK** Cancer Therapeutics Unit at Surrey, is in preclinical development as a combination therapy with poly(ADP-ribose) polymerase (PARP) inhibitors for cancer.[4]

"If CHK2 inhibitors actually do help fight cancer—presumably by disrupting normal DNA repair processes and imposing an insurmountable load of DNA damage—then women would get the dual benefit of oocyte protection plus anticancer activity," said Schimenti. "If ovarian follicles can be protected, young women undergoing cancer treatment will not have to undergo the additional challenge of facing premature menopause. Additionally, reproductive-aged women with cancer would not have to delay therapy until after they cryopreserve oocytes or embryos."

"The reproductive system is highly regulated by a network of hormonal signaling, and it will be very important to assess and compare the long- and short-term effects of CHK2 inhibition on DNA damage repair signaling as well as on hormonal regulation," said Antonio Giordano, director of the **Sbarro Health Research Organization**'s Institute for Cancer Research and Molecular Medicine and of the Center for Biotechnology at **Temple University**.

"It would be ideal to implement conditional knockouts where Chk2 function is preserved up to puberty of mice and then genetically silenced prior to exposure to ionizing radiation or chemotherapy," said Stephen Palmer. "This experimental paradigm more closely resembles the progression of oncologic disease in women, where the disease is diagnosed and therapy applied after oocytes have become follicle enclosed and attained meiotic arrest."

Palmer is CSO of genitourinary company **TocopheRx**, which was launched by **Merck KGaA**'s EMD Serono subsidiary to develop preclinical, oral follicle-stimulating hormone (FSH) agonists to treat infertility.

"There also would need to be thorough analysis of the impact of CHK2 inhibition prior to fertilization on subsequent embryonic and prenatal development in rodents and primates," added Palmer.

"Even if experiments in mice show that offspring of females exposed to both cancer treatment and CHK2 inhibitors do not have a higher mutational load, the same cannot be assumed for humans, so careful testing would be essential. Especially important would be to evaluate the range of chemotherapies that are commonly used and the genotoxicity of each upon CHK2-treated or untreated human oocytes," cautioned Schimenti.

"Ultimately, a clinical study could be designed where a woman of reproductive age has been prescribed a regimen of radiotherapy, is seeking fertility preservation, would elect to have one ovary protected by cryopreservation techniques and the remaining ovary subjected to CHK2 inhibition," Palmer said. "Subsequently, these women would have mandatory preimplantation genetic screening of embryos by karyotype as well as comparative genomic hybridization analysis from blastocyst biopsy. In this manner, the efficacy and safety of CHK2 inhibition can be directly compared to cryopreservation techniques for each patient."

But Palmer thought that the real importance of the study was the ability to push innovation at the interface of varying expertise: fertility and oncology.

"An important outcome of this publication is that it will prompt more conversations between oncologists and fertility specialists, giving patients more potential options," said Palmer.

According to Schimenti, "Fertility preservation in cancer patients, an area of study often called oncofertility, is becoming increasingly important as both men and women have children later in life, thus expanding the number of cancer patients wishing to have children post-treatment. Our work with CHK2 not only provides a promising oncofertility target for fertility preservation but also expands the range of potential targets to other proteins in its signaling pathway."

The Cornell team's findings are not patented.

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