diseases (*7*), whereas their removal from old mice improves health across multiple organ systems and increases life span (*8*).

Why might senescent cells be detrimental in infectious diseases such as COVID-19? Camell *et al.* show that in vitro exposure of senescent human cells to pathogen-associated lipopolysaccharide (LPS) and the S1 subunit of the SARS-CoV-2 spike protein (which mediates cell entry) leads to increased expression of senescence markers and the SASP. Similarly, MHV-infected old (but not young) mice exhibit increased cell senescence and SASP factors, suggesting that pathogen exposure can amplify detrimental inflammation because of senescent cells (see the figure). These findings extend our understanding of the role of viral infection in driving formation of SASP-producing senescent cells (*9*). Notably, SASP factors—especially interleukin-1α (IL-1α)—were found to reduce the expression of interferon-induced transmembrane proteins (IFITMs), a first-line of antiviral defense, as well as increase the expression of the SARS-CoV-2 entry receptor angiotensin-converting enzyme 2 (ACE2) and co-receptor transmembrane protease serine 2 (TMPRSS2) in nonsenescent cells. Hence, SASP secretion predisposes adjacent cells to higher viral infection and poorer innate antiviral responses, in addition to increasing inflammation and tissue damage.

It can be deduced from these findings that the higher the senescent cell burden, the more likely SARS-CoV-2 infection is to lead to severe COVID-19. Older adults (>70 years) and those with chronic conditions such as obesity and diabetes, who already have high amounts of senescent cells and high levels of inflammation (*10*), are most at risk of poor COVID-19 outcomes. The extra "push" from infection is likely to both increase the senescent cell burden and drive senescent cells over a threshold into highly damaging inflammation. Key SASP factors are also those most associated with the lethal cytokine storm that occurs in severe COVID-19 (*2*). Such inflammation is likely to activate complement and clotting cascades, potentially contributing to the high incidence of thrombotic events in severe COVID-19 (*11*) as well as resulting in excess recruitment of neutrophils and natural killer (NK) cells to the lungs, leading to acute respiratory distress syndrome (ARDS).

To test whether senescent cells contribute directly to coronavirus mortality, Camell *et al.* removed senescent cells from infected mice by inducing apoptosis through senescence-specific caspase expression or by treating with senolytic drugs fisetin or a combination of dasatinib and quercetin (D+Q). All approaches resulted in greatly enhanced survival compared with controls. The treatments were accompanied by decreased expression of senescence and SASP markers. Moreover, treated survivors showed improved coronavirus antibody responses; this may simply be because mice survived long enough to mount a full adaptive immune response but may also reflect partial rejuvenation of the immune system through the removal of senescent immune cells.

Senolytic drugs have considerable promise for treating human COVID-19 patients, especially older adults. Fisetin is now in clinical trials in clinically vulnerable adults with COVID-19 (NCT04476953). Moreover, senolytic therapy may also have potential beyond the acute infection phase. Improved physical function has already been reported in patients with idiopathic lung fibrosis, a serious condition with high senescent cell load, after short-term senolytic D+Q treatment (*12*). Therefore, "long COVID" patients suffering from lung fibrosis and difficulty breathing may benefit from senolytic therapy.

In addition to senolytics, other drugs that modify senescent cell behavior may be useful in COVID-19 prophylaxis and treatment (*13*). Inhibitors of mammalian target of rapamycin (mTOR) can act as pleiotropic "geroprotectors," suppressing senescence and the SASP, enhancing antiviral gene expression, and improving adaptive immune responses (*14*). At the low doses that confer geroprotection, mTOR inhibitors are well tolerated in older adults (age 65 to 85 years)—including those with diabetes, asthma, and cardiovascular disease (*15*).

Even with highly effective vaccination campaigns, COVID-19 is likely to become endemic, posing particular dangers to vulnerable older people and those with underlying health conditions. The findings of Camell *et al.* strongly support clinical trials of treatments that target senescent cells in COVID-19 patients, as well as in care homes and long COVID clinics, to improve both resistance to infectious disease and recovery from COVID-19, which if unchecked will contribute to poor quality of life and persistent ill health of COVID-19 survivors. ■

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DEVELOPMENTAL BIOLOGY

The making of an ovarian niche

Ovarian somatic cells are derived in vitro from pluripotent embryonic stem cells

By **Lin Yang** and **Huck-Hui Ng**

Nudging germ cell precursors into functionally mature oocytes and spermatozoa is a key aspect of in vitro gametogenesis and a major challenge in the study of reproductive biology. This process is biologically complex, not only determined by the developmental competency of the germ cell itself but also critically dependent on the gonadal niche. On page 298 of this issue, Yoshino *et al.* (*1*) report the in vitro derivation of fetal ovarian somatic cell–like cells (FOSLCs) from murine pluripotent embryonic stem cells, using a stepwise, directed differentiation strategy to reconstruct in vivo differentiation. These cells sufficiently supported the development of germ cell precursors into functional oocytes that went on to produce viable, fertile mice. The ability to generate and assemble the critical components necessary for oogenesis in the laboratory provides a model system to study the later events of oogenesis, and this may have implications for assisted reproductive technologies.

The preceding decade saw great strides made in understanding early developmental processes in gametogenesis. In the laboratory, methods to direct the specialization of pluripotent stem cells—a renewable cell source—to primordial germ cell–like cells (PGCLCs) were established, first with mouse cells and eventually with human cells. (*2*–*4*). These were successful first steps toward recapitulating gametogenesis in vitro and producing functional germ cells entirely ex vivo.

Further development of mammalian primordial germ cells occurs with their migration to the genital ridges (the location where gonads develop in both sexes) (*5*). In mam-

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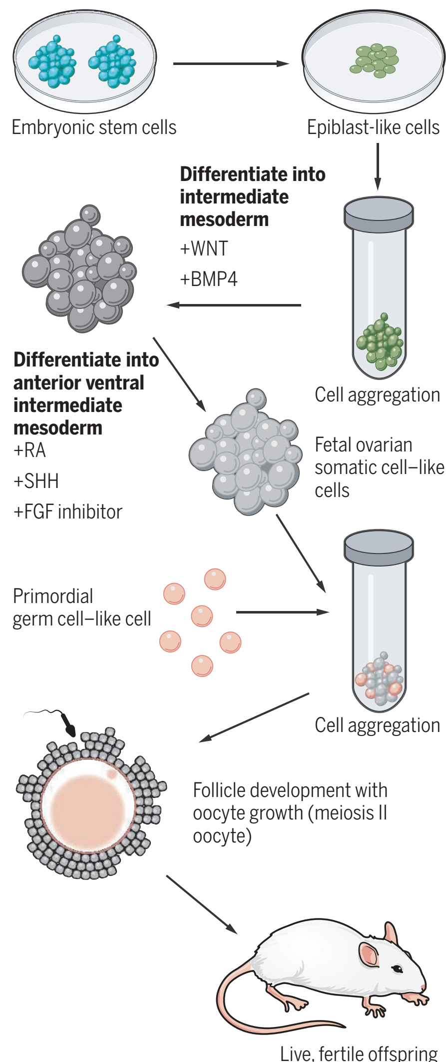
malian females, the developing oocyte is enveloped by ovarian somatic cells (in particular, the granulosa cells) that arise from the fetal gonads. The oocyte releases paracrine growth factors that instruct these support cells to provide nutrients to feed its growing metabolic needs (*6*). This connection is crucial for many developmental milestones, such as the phases of ovarian follicle formation and oocyte entry into meiosis. Mouse pluripotent stem cells have competency to spontaneously differentiate into follicle-like structures around an oocyte-like cell, but this occurs at very low efficiency (*7*, *8*). Without a reliable in vitro source of the support cells, biologists have relied on either transplanting induced PGCLCs back to gonads in vivo or coculturing PGCLCs with dissociated mouse gonad somatic cells to derive functional oocytes (*9*–*11*). Either case requires a preparation procedure that has built-in variability and low scalability, is incompatible with the development of human cell–based systems, and is challenging to manipulate for basic research purposes.

The approach of Yoshino *et al.* relied on using several morphogens [WNT (wingless-related integration site), BMP (bone morphogenetic protein), SHH (sonic hedgehog), and RA (retinoic acid)] to stimulate signaling pathways that guide the differentiation of mouse pluripotent cells (see the figure). Specifically, pluripotent stem cells were coaxed through a differentiation trajectory toward a region of the mesoderm (specifically, the anterior ventral intermediate mesoderm) where the gonads originate. Indeed, the resultant cells captured the cell identities and diversities of the fetal ovaries. Granulosa- and stromal-like cells, as well as less mature precursors, were generated, with transcriptomic signatures (profiles of gene expression) that closely resembled their in vivo counterparts. When FOSLCs were cultured in combination with mouse PGCLCs in three-dimensional aggregates, the "reconstituted ovarioids" supported follicle formation. The authors then achieved the gold standard of in vitro oogenesis—the derivation of healthy, fertile offspring after in vitro oocyte fertilization and transplantation of the embryo into a female mouse.

This technical breakthrough of Yoshino *et al.* holds enormous potential for germ cell research. It allows for fully defined derivation of FOSLCs with substantial improvements in yield and without the need for genetic manipulations. The method will need further refinement—after all, a full recapitulation of all aspects of oogenesis in vitro is still challenging and complex. FOSLCs are less efficient than mouse gonadal somatic cells in generating healthy oocytes, possibly owing to lower proportions of granulosa-like cells among FOSLCs. In addition, it is not yet known how the cytoplasmic contents, or the genetic and epigenetic profiles of in vitro–derived oocytes, match up to those produced in vivo. Nonetheless, FOSLCs and reconstituted ovarioids allow the perturbation of individual molecular factors (for example, specific genes that regulate oogenesis), the investigation of cell type–specific roles of the niche in promoting oocyte maturation, and perhaps the application of bioengineering concepts, much like what has been attempted in tissue and organoid engineering fields, to create more physiological reconstituted ovarioids with higher efficiencies for oogenesis (*12*).

Generation of follicles for in vitro oogenesis

Mouse embryonic stem cells undergo stepwise differentiation into anterior ventral intermediate mesoderm, which gives rise to fetal ovaries. Resulting fetal ovarian somatic cell–like cells are cocultured with primordial germ cell–like cells, which support maturation into oocytes. These are competent to produce live, fertile offspring.



BMP4, bone morphogenetic protein 4; FGF, fibroblast growth factor; RA, retinoic acid; SHH, sonic hedgehog; WNT, wingless-related integration site.

GRAPHIC: N. DESAI/*SCIENCE*

What does this work mean for assisted reproductive technologies in humans, and how far away is the production of autologous, in vitro–derived gametes for clinical use? The proof-of-concept study from Yoshino *et al.* has made clear strides toward enabling in vitro gametogenesis at scale. Similar methods to obtain cells akin to human ovarian somatic cells will no doubt be attempted, but it remains to be seen how transferrable this strategy would be. After all, human gametogenesis occurs on a much lengthier time scale and likely places different requirements on both the germ cells and the supporting niche. For example, primordial germ cell development in humans diverges from that of the mouse in key aspects (*3*). It would be instructive to determine if molecular hallmarks of human oogenesis can be observed in reconstituted ovarioids consisting of human PGCLCs cultured with murine FOSLCs. Additionally, deriving functional gametes in vitro remains inefficient, even in the well-studied mouse model.

The technical challenges for obtaining high-quality cells in humans are thus considerable. Efforts to overcome them will inevitably also come up against ethical conflicts, especially when the developmental competency of later-stage gametes needs to be ascertained. Molecular milestones for oocyte development will have to be used as much as possible, and nonhuman primate models will be particularly useful for demonstrating the final functionality of in vitro–derived gametes in an equivalent nonhuman primate system (*13*–*15*). Such studies will define the contours of the ethical discourse that the scientific community must carefully undertake with the public before any clinical application can be considered and eventually actualized. ■

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