design of AAV vector capsids, retargeting the vector to the LSD brain endothelium.

The surprising finding of this study is that phage panning for two different LSDs—mucopolysaccharidosis type VII (MPS VII) and late infantile neuronal cerlipofuscinosis (LINCL)—resulted in the identification of different peptide epitopes. This finding suggests that the endothelium of a diseased organ has unique characteristics.

This idea is consistent with evolving concepts of endothelial cell biology. Older notions of the endothelium as an inert conduit for flowing blood have given way to the current understanding of endothelium as a distributed organ that covers some 7 m^2 in a typical adult. Molecular and cellular studies have delineated extensive differences among vascular beds in the expression of growth factors, enzymes such as nitric oxide synthase, and cell surface molecules. Thus the heterogeneity of various vascular beds and their ability to change their expression programs and phenotypes as part of a dynamic response to stress or disease states is now well recognized. It is therefore perhaps not surprising that Chen *et al.*[2] have shown that the retargeting of AAV vectors in MPS VII brain is a result of the buildup of chondroitin sulfate, an undigested glycosaminoglycan that accumulates in the presence of the defective enzyme (β-glucuronidase) on the surface of the endothelial cell[2]. The presence of a previously undescribed, unique receptor represents the difference between diseased and healthy endothelium in a specific tissue of interest.

As proof of efficacy, Chen *et al.*[2] showed that the retargeted AAV vectors carrying the therapeutic transgenes specifically target the brain endothelium of affected mice and correct the disease phenotype.

These findings are a breakthrough in terms of gene delivery to the entire CNS. AAV vectors show a tropism for cells in the CNS including neurons and astrocytes and have been used to restore a normal phenotype in mouse models of LSDs, either through direct intraparenchymal injection of brain or by systemic administration early in life, before the BBB is intact[4,5]. Scale-up of these approaches to the clinical arena has proven challenging, given the larger volume of the human brain and low diffusion volumes of injected vector[6]; progress beyond animal models has been quite limited for any gene therapy of the entire CNS, with the exception of the promising results obtained with lentiviral gene transfer for adrenoleukodystrophy[7]. Recent developments with AAV serotypes that either cross the BBB[4] or are transported along neuronal pathways[8] in animal models may also represent future avenues for widespread delivery to the CNS.

Chen *et al.*[2], by using the endothelium rather than the neuron as the target for transduction, have transformed the endothelial cell barrier from a limitation to an opportunity (**Fig. 1**) and have devised a method to allow gene delivery throughout the CNS.

Many steps remain to be completed before application to humans. The normal brain endothelial cell signatures identified by Chen *et al.*[2] differed from those identified earlier in rats[9], so that studies in human cells will need to be done. Depending on the disease and its state of progression at the time of vector administration, therapy may require a mix of vectors, targeted to both normal and diseased endothelium. More extensive evaluation of reversibility of cognitive deficits in mouse models would also be of interest as it relates to human disease[10].

Two contrasting trends in the field, isolation of variants from nature[11] versus development of synthetic vectors based on principles of rational design[12], continue to yield a dizzying array of new AAV serotypes for characterization and testing. This work demonstrates that principles of rational vector design can provide new solutions to problems that have slowed or stopped successful clinical translation of AAV-mediated gene therapy.

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Preserving fertility during cancer treatment

Teresa K Woodruff

Imatinib (trade name Gleevec) preserves fertility in female mice treated with the common chemotherapeutic agent cisplatin. Imatinib seems to block an apoptotic pathway activated by cisplatin in ovarian germ cells (pages 1179–1185). The findings could lead to new ways to protect germ cells from the damaging effects of cancer treatment.

For many individuals with cancer, the decision to protect their fertility from the damaging effects of radiation and chemotherapy is complicated by their age, marital status, the time they have to delay treatment and, sometimes, the uncertainty of surviving their disease. In the past two years, a remarkable alignment between oncologists and fertility specialists has increased access to information about the fertility threats of treatment and the options for fertility preservation[1]. Men and pubertal boys, for instance, now routinely have access to sperm banks and home sperm collection kits[2]. But girls and young women have traditionally been left behind in both the discussion about fertility threats and the options available to them. That, too, is quickly changing.

The current treatment options for young women with cancer who can delay the start of cancer treatment is hormonal intervention followed by cryopreservation of oocytes or fertilized eggs[1]. If the patient has no time to wait, she can opt to have one ovary removed and cryopreserved for her later use[3]. An experimental option for use of the tissue is to transplant pieces of the cryopreserved ovarian cortex back onto her nonfunctional remaining ovary. This process has resulted in the resumption of

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endocrine function and in live human births[3]. The procedure is only possible for individuals with low risk of preexisting contaminating cancer cells in the tissue. For the high-risk group, maturation of eggs within ovarian follicles *in vitro* has been successful in mice and is being adapted to humans[4,5].

The most important breakthrough in this field would be the development of chemotherapeutics that do not harm the resting germ cell or, what I call fertoprotective adjuvant therapy, which would protect the eggs from the damaging effects of drugs. In this issue of *Nature Medicine*, Gonfloni *et al.*[6] examine a combination therapy in the latter category. They show that an approved drug, imatinib, may be the first drug in this class.

One of the chemotherapeutic agents commonly used to treat cancer is cisplatin, which acts by cross-linking DNA and inducing the cell's apoptosis machinery; the effect is the elimination of rapidly growing cancer cells as well as normal cells such as oocytes[7]. The 'off-target' effects of cisplatin cause substantial illness during the drug treatment and infertility long after drug treatment is over[8].

After birth, the primordial oocyte pool, also known as the 'ovarian reserve', lies in a protected dormant state until individual oocytes are called upon to enter the growing population that will result in the release of a single mature oocyte each month[9]. The tumor suppressor protein p63 is expressed in the dormant and growing oocyte, but elimination of the gene does not interrupt normal development of the egg nor does it affect subsequent fertility, at least in mice[10,11].

Previous studies have shown that when an oocyte is challenged with radiation, germ cells die through upregulation of the p63 pathway[12]. Thus, p63 does not act during normal oocyte fate decisions but is upregulated in response to the exceptional damage created by external radiation.

Gonfloni *et al.*[6] now observe a similar effect with cisplatin: p63 serves to eliminate the germ cells that are irreparably injured by iatrogenic chemotherapy, ensuring that the chromosomal damage is not transferred to the subsequent generation.

Gonfloni *et al.*[6] hypothesized that inactivation of p63 during the treatment period would protect the ovarian reserve. The kinase responsible for activating p63 was unknown, and the authors show that the kinase c-Abl is activated in oocytes by cisplatin-mediated DNA damage[6]. Imatinib is a potent inhibitor of c-Abl and, when delivered with cisplatin to immature mice, blocked the immediate appearance of apoptotic oocytes. Furthermore, treatment of immature mice with cisplatin resulted in loss of the primordial follicle cohort and premature sterility in adults. Moreover, treatment of immature mice with imatinib and cisplatin in tandem resulted in normal-appearing follicles in adult ovarian tissue and a normal number of pups born to the treated mothers (**Fig. 1**).

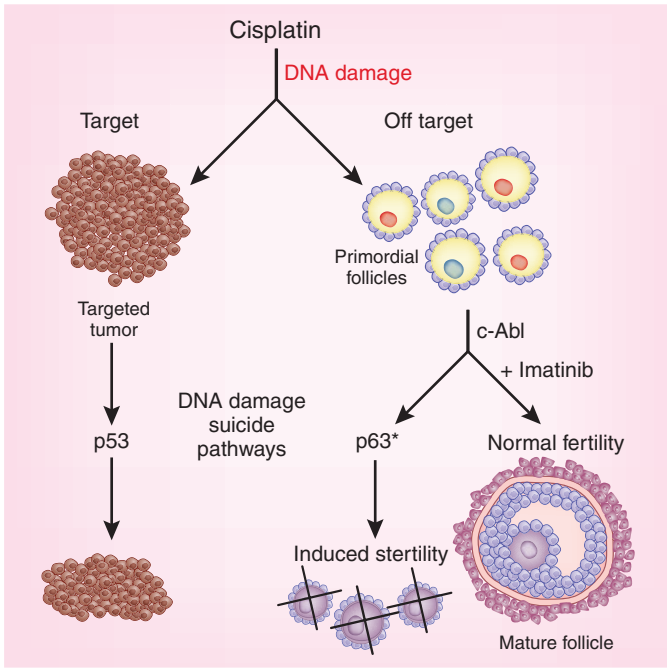

Figure 1 Imatinib protects primordial follicles from cisplatin-mediated death. Cisplatin is a chemotherapeutic agent that kills tumor cells by inducing widespread DNA damage which activates a cascade of signaling pathways leading to cellular apoptosis. Unfortunately, the ovarian germ cells are also targeted by the drug and activate a parallel cell death pathway mediated by the kinase c-Abl and the tumor suppressor gene product p63. Imatinib blocks c-Abl activity and, when delivered with cisplatin, blocks the treatment-induced germ cell death, sparing the ovarian follicles during treatment. Adult mice treated with cisplatin alone become sterile, whereas mice treated with cisplatin plus imatinib are fertile[6].

On the face of it, the new findings seem to be a breakthrough idea. But for individuals with cancer who wish to protect their fertility, selective inactivation of the p63 pathway comes at theoretical costs that must be weighed against other available options. Imatinib treatment does not change how cisplatin works, leading to the question of how healthy the remaining oocytes are. Does the endogenous repair mechanism right the wrong of the DNA adducts in a timely fashion? The lesson from aneuploidy studies is that badly damaged eggs can persist and create embryos, but miscarriage and babies with mild to devastating birth defects are the most common outcomes of chromosomal anomalies[13]. Moreover, imatinib has been shown to amplify the effects of cisplatin in other cancer cell types, leading to some caution and careful assessment of the new off-target sites[14]. Finally, c-Abl is a common kinase activated in tumor suicide pathways[7]. Whether imatinib reduces the effectiveness of cisplatin on the main tumor target must be carefully explored.

These studies are sufficiently tantalizing to inspire additional basic research on the direct and indirect effects of fertoprotective adjuvant treatment, particularly in nonhuman primate models. The preclinical models should parallel clinical studies designed to assess tumor responsiveness and fertility preservation. Such clinical studies, like many in this field, will be challenging; they will require large, longitudinal cohorts to assess menstrual cycles, the time to delivery of offspring and the health of the babies. The health status of the mother and the healthiness of her uterus to bear a pregnancy must all be factored into the analysis.

Despite the hurdles, improved medical management plans for young people with cancer are urgently needed. The use of imatinib as an adjuvant to protect fertility in cisplatin-treated women is an intriguing idea that should be pursued.

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