COMMENTARY

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Combating radiation therapy-induced damage to the ovarian environment

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Radiation is a standard cytotoxic therapy that kills cancer cells through DNA damage. However, in addition to eradicating cancer cells, radiation exposure can have the unintended off-target effect of damaging normal tissues that are within the treatment field. For cancer management requiring abdominal, pelvic or total body irradiation, the ovaries are at risk of substantial exposure. The ovary is a privileged organ as it contains the female germline, and it is highly susceptible to radiation damage [1,2]. Females are born with a fixed number of primordial follicles, which comprise the ovarian reserve and dictate an individual's reproductive lifespan [3]. Because the ovarian reserve is finite and nonrenewable, any factor that damages the follicle pool – such as radiation – can accelerate reproductive aging and lead to premature amenorrhea, subfertility, or even infertility. Such conditions can have significant psycho-social ramifications for patients and may have broader health implications since ovarian hormones are

vital for the functioning of other organ systems (e.g., uterine, bone, cardiovascular, brain, immune) [4]. In addition, exposure to ionizing radiation has the risk of introducing heritable genetic mutations that can impact subsequent generations. Thus, there is an urgent need to examine how existing and emerging prevention, mitigation, and treatment strategies function to protect the entire ovarian milieu from radiation damage.

The ovary consists of several cell types – all of which contribute to the quality and quantity of the germline – but which are also susceptible to radiation damage. The follicle is the functional unit of the ovary comprised of the oocyte and its companion somatic granulosa cells (GCs). Ovarian follicles are embedded in a microenvironment of extracellular matrix and stroma with its complex array of fibroblasts and theca-interstitial, endothelial, and immune cells. Dividing GCs are thought to be the initial target of radiation damage, with evidence of prominent cell death observed within hours

KEYWORDS

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of radiation exposure [5]. Without adequate bidirectional communication maintained between the GCs and the oocyte, follicle survival and/or quality will be compromised. In fact, the oocyte is highly radiosensitive with the LD₅₀ of human oocytes estimated at <2 Gy [6]. The individual oocyte is highly radiosensitive with a value of 0.12 Gy for the Do (reciprocal of the slope on the exponential portion of a survival curve). This translates to sterilization being predicted in 5 and 50% of women whose ovaries receive 2–3 Gy and 6–12 Gy, respectively. The radio-sensitivity of the oocyte depends on its growth phase, with quiescent primordial follicles being more radio-resistant in general relative to larger maturing follicles. In the stroma, radiation damages the vasculature and also results in tissue atrophy and fibrosis [2,5]. Ultimately several factors converge to dictate the extent of radiation damage that occurs in the ovary, including the patient's age and ovarian reserve at the time of treatment, the use of combination therapies, as well as the total radiation dose, irradiated volume, and less critically the fractionation schedule [7].

Protecting normal tissue function from off-target radiation damage is a prime goal of any treatment plan, and general protective approaches include novel radiation beam arrangements, modified fractionation schedules, 3D conformational radiation therapy, and intensity-modulated radiation therapy [5]. In addition to these strategies, specific nonpharmacologic and pharmacologic methods exist or are being actively developed to protect the ovarian environment from damage. For example, use of a lead shield can protect the gonads in female patients undergoing abdominal or pelvic radiation [8]. Although ovarian shielding has been used clinically for decades, accurate placement of pelvic shields is required to ensure that critical orthopedic landmarks are not obscured and that the ovaries are well-positioned within the shielded region [8]. Shielding is not possible, however, in cases where total body irradiation is required or where the ovaries are within the field that must be treated. Ovarian transposition, or oophoropexy, is another nonpharmacologic approach to preserve gonadal function in which ovaries are surgically moved to avoid damage caused by abdominal or pelvic radiation [9]. Where the ovaries are placed depends on the type of cancer, with placement in the paracolic gutters, contralateral to the tumor, and in line with the iliac crests having been reported [9]. Normal endocrine function and fertility occur following

ovarian transposition, but this technique is contraindicated if there is a risk of spreading cancer cells, and up to 14% of the cases have failed to protect ovaries [9].

There are three approaches to pharmacologic radioprotection of normal tissue function: protection, mitigation and treatment [10,11]. Protection refers to prophylactic therapies that begin prior to radiation exposure, and mitigation refers to therapies that are initiated at the time of or shortly following radiation exposure but before any overt tissue damage occurs. Both protection and mitigation strategies are used in an attempt to prevent tissue injury from occurring. In contrast, treatment refers to therapies that are used to treat already existing tissue damage. In the context of fertility preservation and the ovarian environment, protection and mitigation are the most suitable strategies because germ cell depletion is irreversible. The ideal protection and mitigation paradigms are nontoxic and reduce the radiation impact on normal tissues but not on tumor cells.

Promising ovarian protection strategies have been tested in animal models of radiation damage and target apoptosis and growth factor pathways. For example, pretreatment of mice with sphingosine-1-phosphate (S1P), a ceramide antagonist and negative regulator of apoptosis, 2 h prior to radiation exposure protected the ovarian reserve, and irradiated mice were able to produce live offspring [12]. This work was further confirmed in a rhesus macaque model where the ovarian reserve was protected from targeted radiation to the ovary following pretreatment with S1P or its long-acting mimetic (FTY720) [13]. Remarkably, two generations of offspring were obtained in rhesus macaques pretreated with FTY720, providing strong preclinical support for such a strategy [13]. In genetic studies, mice lacking the proapoptotic BH3-only members had a protected ovarian reserve (*Puma*^{-/-} mice) and remained fertile (*Puma*^{-/-} and *Puma*^{-/-}*Noxa*^{-/-} mice) following radiation-induced DNA damage [14]. Thus, directly targeting the BH3-only proteins in the ovary may be an important fertility preservation strategy. Although strategies that block apoptosis protect oocyte cell death, rigorous studies are required to confirm that any DNA damage is effectively repaired in the germline. Otherwise such methods, while useful for restoring endocrine function, will be contraindicated for fertility. Furthermore, it is unclear whether these strategies protect the ovarian stroma in addition to the germ cells. Finally, it must be

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confirmed that these strategies do not also protect cancer cells to the same extent, meaning that no therapeutic benefit is realized.

In addition to inhibition of apoptotic pathways, modulation of growth factor activity may have important radioprotective effects in the ovary. For example, administration of tamoxifen or growth hormone in a rat model for 3 days pre- and post-exposure to total body irradiation (protection and mitigation paradigm) improved reproductive outcomes including follicular development, anti-Müllerian hormone production and even fertility [15,16]. Tamoxifen and growth hormone appeared to enhance IGF-1 expression [15,16]. Interestingly, IGF-1 inhibits radiation-induced apoptosis and PUMA expression in intestinal stem cells, highlighting an important mechanistic interplay between growth factors and BH3-like proteins in radioprotection [17].

Because ionizing radiation generates free radicals that elicit DNA damage, compounds that scavenge free radicals are putative candidates for radiation protection. Amifostine is the first selective-target and broad-spectrum US FDA-approved radio-protector that is used in the treatment of several cancers [18]. Amifostine is an inactive prodrug that is only able to scavenge-free radicals when metabolized into its active form (WR-1065). WR-1065 accumulates preferentially in normal cells relative to cancer cells, providing the therapeutic benefit [18]. In animal models of radiation exposure, protection or mitigation treatment regimens of amifostine inhibited apoptotic pathways in the ovary [19]. Interestingly, while relatively high concentrations of WR-1065 are required at the time of irradiation in order to achieve effective radioprotection via radical scavenging processes, far lower levels present even after irradiation are effective in suppressing mutagenesis and carcinogenesis. Thus, this second mechanism may persist for several hours after administration of the indicated dose of amifostine [20]. These findings, although promising, are still preliminary, and more studies are warranted to understand how efficacious amifostine is in protecting the ovary. Nevertheless, this example demonstrates the value in fully validating the reproductive impact of broad-spectrum radio-protectors that are currently in clinical use for their potential benefit in fertility preservation applications.

As mentioned above, post-radiation pharmacologic treatment for ovarian damage is not relevant if the ovarian reserve is depleted completely. However, if follicles remain, there may

be a benefit in targeting the ovarian stroma. For example, radiation-induced fibrosis is a common long-term side effect of radiation damage in tissues, including the ovary [5,11]. Treatment of radiation-induced fibrosis with various agents, such as copper/zinc superoxide dismutase, manganese superoxide dismutase, or a combination of pentoxifylline and alpha-tocopherol, has resulted in successful fibrotic regression in several systems [11]. Thus, the ovarian stroma may be a critical target for post-radiation treatment, and use of antifibrotic agents may improve the ovarian microenvironment to allow for better residual follicle survival and growth.

Continued development and testing of methods to protect the ovary from radiation damage – in a manner that takes into account a patient's age, cancer diagnosis, treatment regimen, and quality of life goals – is warranted and ongoing. In situations where currently available ovarian radiation protection methods are not suitable, patients may pursue alternative fertility preservation methods, including egg and embryo freezing or ovarian tissue cryopreservation [21]. Integrated discussions with a patient's care team, including oncologists and reproductive endocrinologists, are essential for developing the optimal fertility preservation plan.

In summary, the germ cell has the highest developmental potential within the ovary, but the most successful strategies to minimize radiation damage will go beyond the oocyte to protect somatic components as well. If such a comprehensive approach that also integrates protection, mitigation and treatment could be achieved, patients treated with radiation doses necessary to control their malignancy will still be able to maintain healthy gametes and reproductive function. This goal not only has important implications for oncofertility but also for other scenarios in which healthy women may be exposed to radiation such as space travel or radiologic terrorism.

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