CANCER STEM CELLS

# Survival skills

Several studies have suggested that cancer stem cells (CSCs) might have a role in the radioresistance of some tumours. Michael Clarke and colleagues have provided new data that support this hypothesis, and have also described a mechanism that might explain, at least in part, how some CSCs survive after irradiation.

Haematopoietic stem cells (HSCs) have lower levels of reactive oxygen species (ROS) than their progeny, and Diehn, Cho and colleagues wondered whether this also applied to normal mammary stem cells and breast CSCs. They measured the intracellular concentrations of pro-oxidants using the stain DCF-DA (2′-7′-dichlorofluorescein diacetate)

IMAGESTATE

and found lower levels of ROS in mouse mammary cells enriched for mammary repopulating units compared with more mature progeny.

The authors first examined gene expression data from human breast CSC-enriched populations and non-tumorigenic cells (NTCs). ROS-related genes, specifically those involved in the antioxidant defence system, were expressed more highly in populations enriched for breast CSCs ($CD44^+$, $CD24^{-/low}$, $Lin^-$ cells) compared with NTCs. $CD44^+$, $CD24^{-/low}$, $Lin^-$ cells isolated from surgically resected human breast tumours also had lower levels of pro-oxidants than NTCs, as shown by DCF-DA staining, although there were some differences amongst tumours in the proportion of cells that had low levels of ROS. Interestingly, the authors made similar observations for CSCs and NTCs from head and neck tumours.

To establish a tractable experimental system to analyse this phenomenon, the authors turned to mice expressing mouse mammary tumour virus (MMTV)–*Wnt1*, which develop mammary tumours that are highly enriched for $Thy1^+$, $CD24^+$, $Lin^-$ CSCs. DCF-DA staining again demonstrated that the population of CSCs in these tumours contained more cells with low pro-oxidant levels than the population of NTCs did. Recognizing that free radicals help mediate cell death following irradiation, the authors tested the hypothesis that lower levels of ROS in CSCs impart radioresistance. Immediately after exposure to ionizing radiation, CSCs from MMTV–*Wnt1* tumours had significantly lower levels of DNA damage (both single- and double-stranded DNA breaks) compared with NTCs. More supporting evidence was obtained from intact MMTV–*Wnt1* tumours: there was a twofold increase in the CSC-enriched population compared with NTCs after irradiation, suggesting that the CSCs are more radioresistant. Similar results were found for human head and neck cancer xenografts.

How do CSCs maintain low levels of ROS? Because enhanced ROS defence mechanisms might have a role in this process, the authors examined glutathione, an antioxidant that has been implicated in radioresistance of cancer cells. Using single-cell quantitative reverse transcription PCR, they found that *Gclm* and *Gss* (two glutathione biosynthesis genes), and *Foxo1* (which encodes a transcription factor that regulates anti-ROS gene expression in HSCs) were overexpressed in many cells in the CSC-enriched population from MMTV–*Wnt1* tumours. Finally, pharmacological manipulation of ROS levels in NTCs and CSCs *in vitro* indicated that NTCs can be radioprotected by treatment with the nitroxide antioxidant tempol and that CSCs can be radiosensitized by treatment with L-*S*,*R*-buthionine sulphoximine, which inhibits the enzyme that catalyses the rate-limiting step in glutathione synthesis.

These data provide compelling evidence that some tumours contain CSCs that can withstand radiotherapy owing to enhanced ROS defence mechanisms that lead to reduced levels of ROS. Inhibiting these defence mechanisms might help improve the efficacy of radiotherapy for some tumours.

*Sarah Seton-Rogers*

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