

## Cellular radiation effects in Belfast

# Spreading the Word

Susanne Burdak-Rothkamm and her colleagues from Kevin Prise's group in Belfast study the bystander effect that occurs when non-irradiated cells receive signals from neighbouring irradiated cells. Recently, they have discovered that two certain cell cycle arrest modulators are key players. In the long run, these findings will hopefully help to improve the targeting of radiotherapy.

**I**onising radiation, such as the X-rays and  $\gamma$ -rays that are used in radiotherapy, causes a range of DNA damage, of which double-strand breaks (DSB) are thought to be the most relevant. DSBs are dangerous for cells because if the subsequent repair is faulty or incomplete it can lead to genome rearrangements. DNA damage leads to cell cycle arrest, giving the cell time to repair the damage before replication continues. If this repair attempt is unsuccessful, however, the cell will undergo programmed cell death (apoptosis).

### Innocent bystanders

The two proteins, ATM (Ataxia telangiectasia and Rad3 related) and ATR (Ataxia telangiectasia mutated), are responsible for the induction of cell cycle arrest. ATM responds to DSBs and ATR to stalled replication forks. A signal transduction cascade then leads to cell cycle arrest, repair or apoptosis. Some of the downstream targets of ATM and ATR are tumour suppressors such as p53 (the master regulator of apoptosis) and BRCA1 (mutated in breast cancer).

Ionising radiation-induced effects in cells that have not directly been irradiated are called bystander effects. Bystander cells show some signs of DNA damage, as if they too had been directly irradiated. The irradiated cells seem to communicate the radiation event to other cells. It is not yet completely clear what kind of damage occurs in bystander cells. Effects that have been looked at are sister chromatid exchanges, nuclear foci of phosphorylated Histone H2AX ( $\gamma$ -H2AX) as a marker for DSBs, micronucleation, cell death, and mutagenesis. The bystander effect can occur

in cells that are in direct contact with irradiated cells but also over some distance or transferred through conditioned media.

Susanne explains that "the first studies on the radiation induced bystander effect have been published in the 1990s. A clastogenic effect of plasma from patients receiving radiotherapy on unirradiated cells was described much earlier." Several studies going back as far as the 1950s describe an induction of chromosome breakage in cultured cells after incubation with blood plasma from patients that had either received high-dose radiotherapy or accidental radiation.

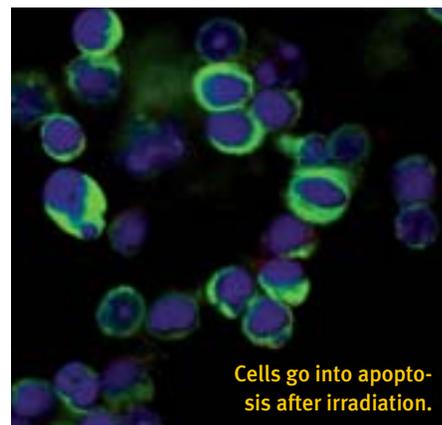
Susanne Burdak-Rothkamm and her colleagues in Kevin Prise's group have studied the bystander effect for several years.



Susanne Burdak-Rothkamm

Susanne worked at the University of Saarland, Germany, before she came to the UK to join Kevin Prise's group in December 2003. The group worked at the Gray Cancer Institute, Northwood, until, in summer 2007, Kevin Prise became Professor of Radiation Biology at Queen's University, Belfast. Susanne spent most of her time finishing experiments at their former lab until August this year, when she took up her clinical training again and started work as a specialty registrar in histopathology at Wycombe Hospital, northwest of London. "I have very much enjoyed working in Kevin's group with many very good colleagues. The new Centre for Cancer Research & Cell Biology at Queen's University, where the group is located, offers state-of-the-art facilities and a lot of potential for collaborations. I was really sorry to leave."

It was believed that direct damage to DNA through a hit to the nucleus would be needed to trigger bystander effects. Surpris-



ing findings showed that cytoplasmic irradiation and very low doses of radiation were sufficient. The effect seems to be independent of the level of DNA damage and also independent of the fate of the signalling cell. If it lives or dies, it still lets other cells know about the assault. It is still unclear which mechanism in the signalling cell leads to the bystander effect but, as Susanne mentions, "There is a most recent publication from Hagelstrom *et al.* in *Oncogene* focussing on the role of ATM and DNA-PK in the generation of the bystander signal."

### Calamitous co-cultures

Several different mechanisms are being considered when it comes to how the signal is transferred from the irradiated cell to the recipient. In co-cultured cells, gap junctions are likely to be involved. But in experiments where media are transferred from irradiated to non-irradiated cells, soluble molecules seem responsible for the signalling.

Susanne explains, "A current hypothesis assumes that ROS cause DNA damage in bystander cells. The prolonged generation of ROS is thought to be mediated by cytokines (e.g. TGF $\beta$ 1). The pathways may be cell type dependent."

### The fate of the survivors

In a paper published last year (*Oncogene* 26: 993-1002) Susanne and her colleagues have shown that ATR is involved in the induction of  $\gamma$ -H2AX foci in bystander cells using glioma cells and astrocytes. They used either a technique transferring conditioned media from irradiated cells to non-irradiated cells or co-cultures of irradiated with non-irradiated cells to induce the effect. They observed  $\gamma$ -H2AX foci formation predominantly in S-Phase cells and postulated the hypothesis that DNA damage in bystander cells interferes with the replication fork, resulting in stalled replication forks which lead to the recruitment of ATR. In their current paper (*Cancer Res.* 68:

7059-65) they describe how ATM is phosphorylated in an ATR-dependent manner in bystander cells. They show co-localisation and induction of nuclear foci for  $\gamma$ -H2AX, ATR, phosphorylated ATM and other downstream effectors like BRCA1. All these pathways probably lead to cell cycle arrest, DNA repair and apoptosis.

The difference in the response of targeted versus bystander cells to irradiation seems to be that "directly irradiated cells rely on DNA-PK and ATM for the repair of the induced DNA damage. The DNA damage in bystander cells seems to be predominantly recognised by ATR with subsequent activation of ATM." This important finding, made by Susanne and her colleagues, means that ATR/ATM inhibitors have different effects on cell destruction by radiation depending on whether they are directly targeted or bystander cells. This immediately has implications for radiotherapy.

#### How to inhibit the bystander response

Studying bystander effects further will hopefully lead to a way of modulating cell survival and destruction in radiotherapy towards a better treatment outcome. "A better understanding of bystander signalling pathways may provide a rationale for the differential modulation of cell survival/cell killing in targeted and non-targeted cells with molecular targeted drugs, e.g. increase tumour cell killing (directly targeted) but decrease the damage to surrounding normal tissue (bystander). In order to do this in a safe way, we first have to establish the fate of surviving bystander cells, the kind of damage they may accumulate and the probability of genomic instability in bystander cells," emphasises Susanne.

There are already trials taking place that aim to sensitise tumours to radiother-

apy by systemic treatment with ATM inhibitors. Tumour cells treated with these inhibitors would not be able to repair the damage caused by radiation and would die. If a tumour is treated with a drug then surrounding cells will also be affected. In this case no bystander response (inducing repair or cell death) will follow DNA damage after receiving signals from irradiated neighbouring cells. "The increased survival of bystander cells upon treatment with ATR/ATM inhibitors bears the chance of bystander cells having accrued DNA damage or genomic instability and which may later give rise to secondary cancers."

#### From head to spleen

It is difficult at this stage to estimate the risk that bystander effects play in promoting secondary cancers in cells that have previously accumulated DNA damage. Susanne emphasises that their study, "focussed on the response of the recipient cells to the bystander signal and aimed at investigating the therapeutic potential for a differential modulation of targeted and non-targeted radiation effects but also flagging up potential risks. It will hopefully be a good basis for future studies. There is huge potential for future studies as in an *in vivo* model bystander effects distant from the field of radiation have recently been reported by Koturbash *et al.* (*Mutat. Res.* 642: 28-36)." In several studies, this group has shown a bystander effect in the spleens of mice that have received an X-ray radiation incident to the head.

These discoveries illustrate that there still remains a lot to be discovered about the fascinating bystander effect and that we can still learn more about how cells in the body communicate with each other.

ANDREA HERB

## ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER

