Supporting information

Communication of Radiation-induced signals in vivo between DNA Repair Deficient and Proficient Medaka (Oryzias latipes)"

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Materials and Methods: Additional information

Medium transfer

The technique used has been described in detail in (1). Briefly, medium was poured off donor flasks containing explants from the different experimental groups. This was filtered through a 0.22 □ filter, to ensure that no cellular material could still be present in the transferred medium. Culture medium was then removed from the flasks designated to receive the explant medium and the filtrate was immediately added to these recipient flasks. Standard plating efficiency controls were also set up. The explants from which the medium had been harvested were incubated for a further 14 days in Clonetics serum free Keratinocyte Growth Medium (Clonetics Corp. San Diego, USA)

Immunostaining for Bcl 2 and cMyc activity in Explant cultures

Explant cultures and reporter colonies were fixed in 10% unbuffered formalin and stored at 21oC until processed. Processing always took place within 7 days of fixation. The culture was processed in situ on the flask bottom. Cultures were stained for expression of bcl-2 and cmyc. The bcl 2 and cmyc primary antibodies used were mouse monoclonals obtained from DAKO laboratories. All were recommended for immunohistochemistry with mouse tissues but have been previously shown to work for rainbow trout and zebrafish (2,3,4). Immunochemistry was performed using an appropriate Vectastain ABC kit (Burlingame, USA). Diaminobendizine (DAB) was used to express the positive reaction and cultures were lightly counterstained with Mayers Haematoxylin. At least

three flasks are stained for each antibody and over 200 cells scored over 5 fields using an Olympus image analysis system (IMAGE PRO). The detection threshold for positivity was set using positive control sections from positive tissue blocks obtained from the Cell Pathology service. Positive and negative control sections were carried with every immunocytochemistry run to correct for run variability. This method was established in the laboratory several years ago and is discussed fully in (5).

Apoptotic cells (Figure S2) were identified using morphology (6) and cmyc staining. 200 cells were scored over 5 random fields for each explant using IMAGE PRO software.

Clonogenic reporter cell lines

The reporter cells (HPV-G or HaCaT) are adherent epithelial cells derived originally from human skin primary culture. HPV-G were established from a foreskin then immortalised by the HPV virus (7) They were obtained as a gift from Prof J DiPaolo and have been used in our laboratory as a reporter system for bystander signal production in a wide range of experiments (e.g. 8,9). The cells are non-tumourigenic, have about 30% wild-type p53 expression (10) and have a normal epithelial pattern of cobblestone density inhibited cell growth. They are used because when exposed to autologous medium harvested from irradiated cells, they give a stable bystander effect of approximately 40% reduction in plating efficiency over a very wide range of doses and exposure conditions (11). This allows comparison of bystander inducing signal strengths even when the HPV-G cells are exposed to signals from other cell lines or from explants. The line was maintained in T75 flasks (NUNC Inc, Uden, Netherlands) and subcultured into T25

flasks (40ml volume) for experiments. HaCaT cells are also human keratinocytes. They were established from a normal skin biopsy of an adult male patient with malignant melanoma (12). They have a mutation in one copy of the p53 gene and a deletion in the other copy. Thus they express mutant p53. When exposed to medium from autologous but irradiated HaCaT cells they show a bystander effect of similar magnitude to that observed with HPV-G cells. The culture conditions are similar.

Clonogenic assay for bystander activity using keratinocytes as reporters:

Flasks of cells which were 85-90% confluent and that had received a medium change the previous day were chosen. Cells were removed from the flask using 0.25 %w/v trypsin/ 1mM EDTA solution (1:1). When the cells had detached they were resuspended in medium, and an aliquot was counted using a Coulter counter model Z2 set at a threshold calibrated for the cell line using a haemocytometer. Flasks were seeded with 500 cells and left to attach for 6hrs before receiving the medium harvested from the explants. Medium was harvested 48hrs after the explants were set up.

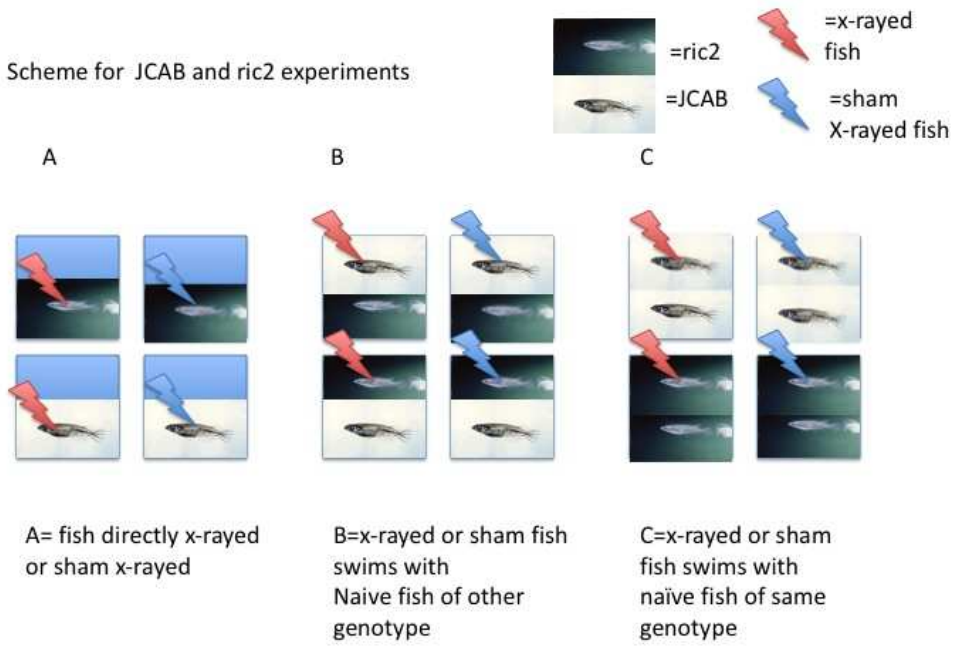
Scheme for JCAB and ric2 experiments
=ric2
=JCAB
=x-rayed fish
=sham X-rayed fish
A
B
C
A= fish directly x-rayed or sham x-rayed
B=x-rayed or sham fish swims with Naive fish of other genotype
C=x-rayed or sham fish swims with naïve fish of same genotype

Figure S1

Senescent cell and apoptotic bodies

Figure S2

References

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