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Short communication

Predicted ionisation in mitochondria and observed acute changes in the mitochondrial transcriptome after gamma irradiation: A Monte Carlo simulation and quantitative PCR study

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ABSTRACT

It is a widely accepted that the cell nucleus is the primary site of radiation damage while extra-nuclear radiation effects are not yet systematically included into models of radiation damage.

We performed Monte Carlo simulations assuming a spherical cell (diameter 11.5 μm) modelled after JURKAT cells with the inclusion of realistic elemental composition data based on published literature. The cell model consists of cytoplasm (density 1 g/cm^3), nucleus (diameter 8.5 μm; 40% of cell volume) as well as cylindrical mitochondria (diameter 1 μm; volume 0.5 μm^3) of three different densities (1, 2 and 10 g/cm^3) and total mitochondrial volume relative to the cell volume (10, 20, 30%). Our simulation predicts that if mitochondria take up more than 20% of a cell's volume, ionisation events will be the preferentially located in mitochondria rather than in the cell nucleus.

Using quantitative polymerase chain reaction, we substantiate in JURKAT cells that human mitochondria respond to gamma radiation with early (within 30 min) differential changes in the expression levels of 18 mitochondrially encoded genes, whereby the number of regulated genes varies in a dose-dependent but non-linear pattern (10 Gy: 1 gene; 50 Gy: 5 genes; 100 Gy: 12 genes).

The simulation data as well as the experimental observations suggest that current models of acute radiation effects, which largely focus on nuclear effects, might benefit from more systematic considerations of the early mitochondrial responses and how these may subsequently determine cell response to ionising radiation.

1. Introduction

Most studies of cellular responses to ionising radiation are centred on the nuclear DNA, whereby the DNA repair processes, rather than the damage directly, are used as proxy read-outs to determine the extent of nuclear DNA damage (Aziz et al., 2012). However, significant effects of ionising radiation on mitochondrial functions (Hwang et al., 1999; Yukawa et al., 1985), mitochondrial oxidative stress (Hosoki et al., 2012; Kobashigawa et al., 2011; Motoori et al., 2001; Tulard et al., 2003) and apoptotic pathways (Belka et al., 2000; Chen et al., 2003; Leach et al., 2001; Zhao et al., 1999) have been reported. Indeed, some experimental observations indicate that the mitochondrial genome may be more susceptible to damaging effects by gamma irradiation than the nuclear genome (Gong et al., 1998; May and Bohr, 2000; Morales et al., 1998), possibly by virtue of the greater likelihood of mitochondria suffering oxidative damage (Yakes and Houten, 1997). In addition to direct radiation effects on mitochondria, mitochondria dysfunction may exert an indirect influence on the nucleus and perpetuate radiation-induced genomic instability (Kim et al., 2006a, 2006b; Miller et al., 2008).

Monte Carlo track structure simulations (Zaider et al., 1983) can be used to estimate likely regions of radiation damage within the cell (Alard et al., 2002; Chauvie et al., 2007; Miller et al., 2000). To date, however, track structure simulations have mostly focused on predicting the occurrence of single or double strand breaks in nuclear DNA as a result of physical processes leading to ionisation formation (Grosswendt, 2005; Nikjoo and Goodhead, 1991; Nikjoo et al., 1999). Here, we employ Monte Carlo simulation and develop a more realistic cell model containing both cell nucleus and mitochondria, as well as currently available data on the elemental concentration in mitochondria (Ernster and Lindberg, 1958; Taylor et al., 1999), in order to predict regions mostly likely to be subject to damage from ionisation formation.

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We base our model for the simulation on the human leukemic JURKAT cell line, which has previously been used to investigate radiation effects *in vitro* (Cataldi et al., 2009; Syljuasen and McBride, 1999; Vigorito et al., 1999). In suspension, the cells are of near-spherical shape (Rosenbluth et al., 2006; Roskams and Rodgers, 2002) and are well characterised with respect to the relative volume taken up by mitochondria (Cataldi et al., 2009; Chaigne-Delalande et al., 2008; Kawahara et al., 1998; Ueda et al., 1998; Yasuhara et al., 2003), and their geometry can, therefore, be modelled relatively easily.

PCR or quantitative PCR (qPCR) has previously been used to measure changes in gene expression levels after radiation exposure (Gong et al., 1998; Gubina et al., 2010; Kulkarni et al., 2010). To validate *in vitro* the predictions made by our Monte Carlo simulation that mitochondria respond to radiation exposure, we quantify by qPCR in JURKAT cells the acute changes in the expression levels of mitochondrial electron transport chain genes as well as mitochondrial transfer RNAs and ribosomal RNAs, in response to a single radiation dose ranging from 10 to 100 Gy.

In the present study, we introduce for the first time a model that specifically includes the mitochondrial compartment, and makes realistic assumptions in regard to the content of those atomic elements in mitochondria that are important for predicting the likely localisation of ionisation events. The qPCR results provide biological evidence that mitochondria are involved in the early cell response to gamma radiation.

2. Material and methods

2.1. Monte Carlo simulation

Simulations were performed using the open-source, general-purpose Monte Carlo radiation transport simulation toolkit Geant4 version 9.4.p02 (Agostinelli et al., 2003; Allison et al., 2006).

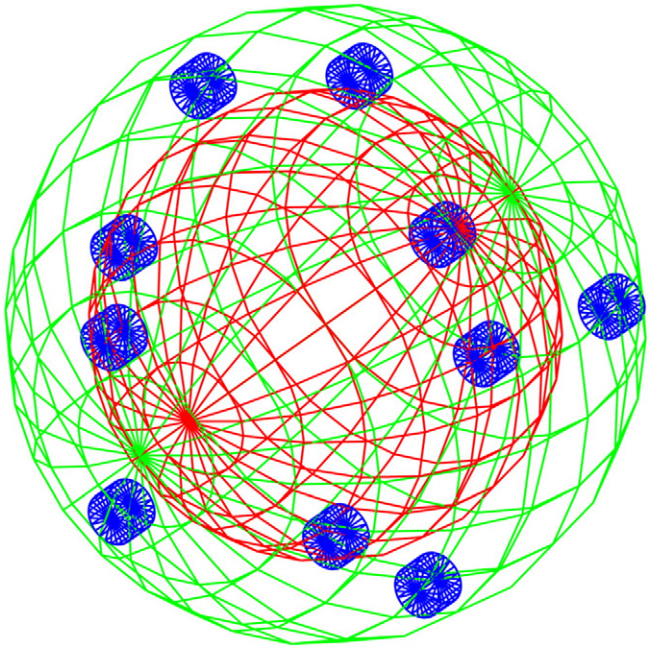

Fig. 1. Illustration of the geometry used in the Geant4 Monte Carlo radiation transport simulation. The cell is filled with a realistic cytoplasm material. Two specific cell organelles are modelled: nucleus (red sphere) and mitochondria (blue cylinders) randomly distributed throughout the cell. The geometry is realistically based on the spherical cell shape of JURKAT cell in suspension.

2.1.1. A compartmental cell model

A single JURKAT cell was modelled to predict the distribution of energy deposition and ionisation events within 3 different cellular components, namely the cell cytoplasm, nucleus and mitochondria (Fig. 1).

The cell was modelled as a sphere of diameter 11.5 μm containing a centrally located spherical nucleus with a calculated diameter of 8.5 μm (Rosenbluth et al., 2006). Individual mitochondria were modelled as cylinders with a diameter of 1 μm and total volume of 0.5 μm^3 (Roskams and Rodgers, 2002), randomly distributed throughout the cytoplasm. The cell was filled with a uniform cell cytoplasm material of density 1 g/cm^3 and a chemical mass fractional constituent of H (8.93%), O (58.09%), C (19.97%), N (8.47%) and P (4.54%) (Alard et al., 2002). Similarly, the nucleus was filled with a uniform material of density 2.0 g/cm^3 and a mass fractional composition of H (10.64%), O (74.5%), C (9.04%), N (3.21%) and P (2.61%) (Alard et al., 2002). Using published electron-microscopic data (Cataldi et al., 2009; Chaigne-Delalande et al., 2008; Kawahara et al., 1998; Ueda et al., 1998; Yasuhara et al., 2003), indicates that in un-stimulated JURKAT cells, a cell type that is near-spherical, mitochondria take up approximately 20–30% of the cell volume. Additionally, it has been reported that the total mitochondrial volume in mammalian cells is ~13% (Kilby, 1979), and that of a lymphocyte is also in a similar range (Mayhew et al., 1979). For this reason, 3 different mitochondrial volumes were investigated: 10%, 20% and 30% of the total cell volume. The density and chemical mass-fraction constituents for mitochondria is yet to be fully determined, however, the presence of heavy ions (*e.g.* Ca, Mg and Na) have been reported in mitochondria (Ernster and Lindberg, 1958; Taylor et al., 1999). We investigated 3 different mitochondrial densities in the simulations, 1, 2 and 10 g/cm^3 (based on net wet weight calculations in (He et al., 2010)) and the chemical mass fractional composition was set to H (10.64%), O (71.5%), C (9.04%), N (3.21%), P (2.61%), Na (1%), Ca (1%) and Mg (1%) as a first approximation for all cases. The cell was modelled at the centre of a liquid water 1.5 ml volume cylinder.

Photons were randomly selected from a ^{60}Co source at a distance of 9 mm from the cell centre and emitted across the entire cell volume for each simulation case. The photon interactions within the volume were modelled with the Geant4 Low Energy Electromagnetic Package based on the Livermore libraries, valid down to particle energies of 250 eV. The physics processes included the photoelectric effect, Compton scattering, Rayleigh scattering and pair production for photons. Additionally, secondary electrons were tracked and the processes activated included ionisation, bremsstrahlung and multiple scattering. Secondary electron ionisations were modelled and the low energy cut-off for the production of secondary particles was set to 250 eV. A total of 1.9976×10^8 incident photons (energies 1.33 MeV and 1.1732 MeV sampled from a ^{60}Co source) were simulated for each case. The absorbed dose as well as the total number of ionisations occurring within each cellular component was calculated.

2.2. Cell irradiation and RT-qPCR

2.2.1. Sample preparation

Wild type JURKAT cells were maintained at 37 °C with 5% CO_2 in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin until the day of the experiment. For testing the radiation effect on nucleic acids within cells, cells were irradiated at a density of 0.16×10^6 in 1 ml medium (as described above) in a 1.5 ml-Eppendorf tube at room temperature.

2.2.2. Gamma irradiation

JURKAT cells were gamma irradiated, at room temperature (21 ± 2 °C in this study), using a ^{60}Co irradiator (GammaCell 220) at 10, 50, 100 Gy which is the total dose range generally delivered in radiation therapy or used in radiation experiments (see Section 3.2).