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Mathematical modeling of growth and death dynamics of mouse embryonic stem cells irradiated with γ-rays

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HIGHLIGHTS

- Mathematical model of growth and death dynamics of irradiated cells.
- Model parameters are derived from the experimental data.
- The model estimates the duration of the different phases of the apoptotic process.
- Quantitation of the increased probability to undergo apoptosis after treatment.

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ABSTRACT

Following ionizing radiation, mouse embryonic stem cells (mESCs) undergo both apoptosis and block at G2/M phase of the cell cycle. The dynamics of cell growth and the transition through the apoptotic phases cannot be directly inferred from experimental data, limiting the understanding of the biological response to the treatment. Here, we propose a semi-mechanistic mathematical model, defined by five compartments, able to describe the time curves of untreated and γ-rays irradiated mESCs and to extract the information therein embedded. To this end, mESCs were irradiated with 2 or 5 Gy γ-rays, collected over a period of 48 h and, at each time point, analyzed for apoptosis by using the Annexin V assay. When compared to unirradiated mESCs, the model estimates an additional 0.2 probability to undergo apoptosis for the 5 Gy-treated cells, and only a 0.07 (not statistically significantly different from zero) when a 2 Gy-irradiation dose is administered. Moreover, the model allows us to estimate the duration of the overall apoptotic process and also the time length of its early, intermediate, and late apoptotic phase.

1. Introduction

Embryonic stem cells (ESCs) derive from the inner cell mass of a blastocyst and represent an important *in vitro* model system to study the effects exerted by ionizing radiations on a remarkable type of pluripotent stem cells, i.e., those that contribute to the formation of a new individual (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1998). When compared to terminally differentiated cells, ESCs exhibit a more strict control on their genome integrity (Saretzki et al., 2004; Lee et al., 2007; Stambrook, 2007; Saretzki et al., 2008; Tichy and Stambrook, 2008) and, following ionizing radiation treatment, which induces single and double DNA strand breaks, they become more responsive to apoptotic signals triggered by DNA damage (Saretzki et al., 2004; Roos et al., 2007; Saretzki et al., 2008). In mouse ESCs (mESCs), the lack of the G1/S checkpoint (Aladjem et al., 1998; Mailand et al., 2000; Bartek and Lukas, 2001; Hong and Stambrook, 2004; Roos et al., 2007; Rebuzzini et al., 2012), which is commonly involved in the DNA repair response, likely explains the elimination from the population of those cells with a high burden of unrepaired single or double

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strand breaks (Van Sloun et al., 1999; Roos et al., 2007; Rebuzzini et al., 2012) in order to ensure the correct transmission of undamaged DNA (Stambrook, 2007; Tichy and Stambrook, 2008).

We have recently shown that mESCs exposed to a therapeutic 2 Gy irradiation dose undergo, after 24 h culture, both apoptosis and a block of the cell cycle at the G2/M phase (Rebuzzini et al., 2012). This block is released between 48 and 72 h post-irradiation, when the frequency of cells within each cell cycle phase and apoptosis returns to control levels. On the other hand, at the higher irradiation dose of 5 Gy, even after 96 h culture, mESCs still undergo cell death and maintain a clear evidence of a G2/M block. After 96 h at both irradiation doses, the surviving cells maintain their unique self-renewal capacity, fast cell cycle and undifferentiated status (Rebuzzini et al., 2012). Overall, these results indicate that following ionizing radiations mESCs undergo complex dynamics of cell growth and death, complicating the understanding of the biological response to the treatment.

To this regard, mathematical modeling is becoming an effective approach to unravel complex biological systems (Voit et al., 2008), to formulate hypotheses on mechanisms of cell growth (Zheng et al., 2013; Liu et al., 2013; Hadjiandreou and Mitsis, 2014) and differentiation (Lefevre et al., 2013; van der Wath et al., 2013), and to develop new experimental assays (Byrne et al., 2011). Depending on the level of the mechanistic description of the underlying biological processes, mathematical models can be grouped into three main categories: (i) descriptive/empirical models, (ii) mechanistic models and (iii) large scale/system biology models (Simeoni et al., 2013). To avoid complex models characterized by a large number of parameters that cannot be estimated from the bulk of experimental data, semi-mechanistic models, which lay in-between empirical and mechanism-based approaches, have been proposed and widely adopted (Thakur, 1991; Simeoni et al., 2004; Ribba et al., 2012; Terranova et al., 2013; Carson and Cobelli, 2013). These semi-mechanistic models, which incorporate only some mechanistic assumptions, are able to describe the considered biological system by few identifiable and biologically relevant parameters.

Here, we propose a semi-mechanistic model to obtain quantitative parameters that describe the dynamics of cell growth and death of mESCs treated with γ-rays. To this end, mESCs were irradiated with 2 or 5 Gy γ-rays, collected after 1, 4, 8, 10, 14, 16, 20, 24 or 48 h from the treatment and, at each time point, analyzed for apoptosis by using the Annexin V (AnV) assay. This assay is based on the detection of viable cells and of three main events occurring along the apoptotic pathway (Fig. 1). An early event is the externalization of phosphatidylserine (PS), an aminophospholipid, that is translocated from the inner to the outer side of the plasma membrane, whereas in non-apoptotic cells it remains facing the cytosol (Fig. 1a). AnV, a binding protein with strong and specific affinity for PS, evidences the PS translocation (Fig. 1b). In a further step of apoptosis, the cell membrane loses its integrity allowing the penetration of the DNA-binding fluorochrome propidium iodide (PI) (Fig. 1c). Then, as a consequence of the plasma membrane disaggregation, the late stage of apoptosis is characterized by the loss of AnV signals and the maintenance of the PI fluorescence (Fig. 1d). Using this assay, we described the presence of four subpopulations: (i) viable cells (negative to both AnV and PI, AnV^-/PI^-); (ii) early apoptotic cells (positive to AnV, but negative to PI, AnV^+/PI^-); (iii) intermediate apoptotic cells (positive to both AnV and PI, AnV^+/PI^+); (iv) late apoptotic cells (positive to PI and negative to AnV, AnV^-/PI^+).

2. Material and methods

2.1. Maintenance of mouse embryonic stem cell line

The mESC line R1 (provided by Dr. Nagy, Samuel Lunenfeld Research Institute Mount Sinai Hospital, Toronto, Canada) was expanded in KO-DMEM supplemented with 0.5% penicillin–streptomycin solution, 0.1 mM β-mercaptoethanol, 2 mM L-glutamine, 1 × non-essential amino acid solution, 15% ESC Qualified Fetal Bovine Serum (all from Life Technologies) and 500 units/ml of ESGRO® –leukemia inhibitory factor (LIF) (Chemicon). Cells were routinely passaged enzymatically every 2–3 days alternating a passage on STO feeder cells (American Type Culture Collection CRL-2225) with two passages on gelatin-coated 100 × 20 mm Petri dishes, using 0.05% trypsin-EDTA solution (Life Technologies), pre-warmed at 37 °C.

2.2. Ionizing radiation treatment

About 12×10^6 cells were irradiated in a T75 flask in suspension with a ^{60}Co Teletherapy Unit (CGR/VARIAN ALCYON II) at 2 or 5 Gy dose and at a rate of about 0.9 Gy/min. Irradiation was carried out in a field of 32 × 32 cm by placing the flasks on an acrylic phantom filled with warm water at 37 °C, with the cells at a distance of 80 cm from the source. A dosimetric system was used to measure the nominal dose, traceable to a secondary calibration standard (PTW "farmer type" ionization chamber of 0.6 cc, Unidos PTW electrometer). The global uncertainty for nominal doses, including

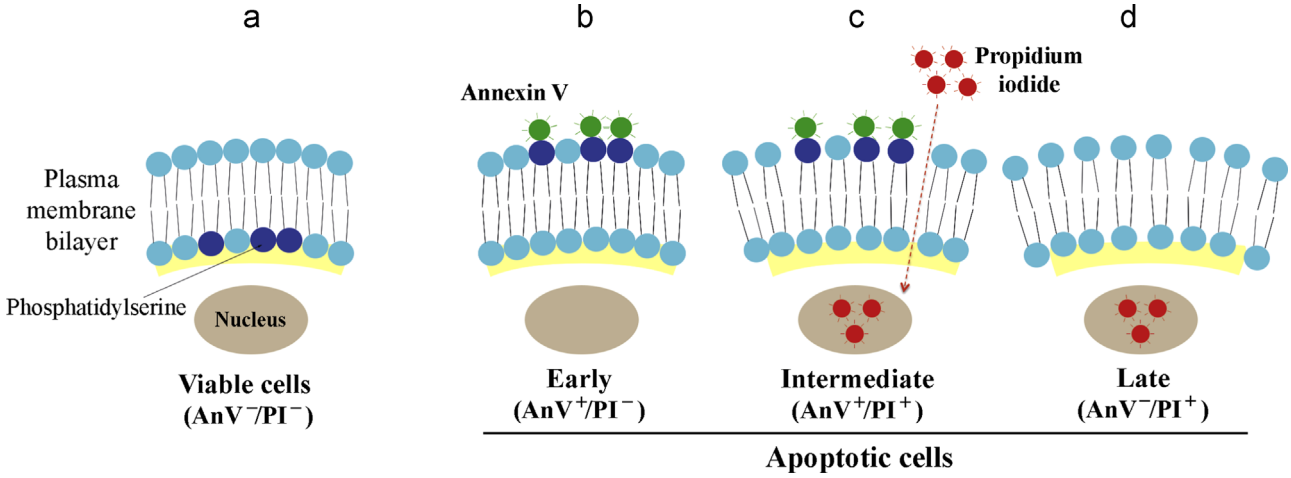

Fig. 1. Main apoptotic events observed when using the AnV assay: (a) viable cells; (b) externalization of phosphatidylserine (PS), translocated from the inner to the outer leaflet of the plasma membrane, as evidenced by the AnV binding; (c) penetration of the DNA-binding fluorochrome propidium iodide (PI); (d) plasma membrane disaggregation, loss of AnV signals and maintenance of the PI fluorescence.