Relationship between spontaneous or radiation-induced apoptosis and telomere shortening in G₀ human lymphocytes

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ABSTRACT

To examine the correlation between spontaneous or radiation-induced apoptosis and telomere shortening, G₀ human peripheral blood lymphocytes were irradiated with X-rays and analyzed for viability, apoptosis, and telomere length. Part of the lymphocytes was kept under liquid-holding conditions for 48 h, and then loaded onto Ficoll-Paque medium to separate apoptotic (high-density) from normal (normal-density) cells. Then all samples were examined for the same three end-points. To determine whether expression of p53 influences the telomere shortening associated with a spontaneous or radiation-induced apoptotic process, the lymphocytes were also analyzed for expression of p53 at 0 and 48 h recovery times (non-irradiated and irradiated samples) and after 2 weeks in liquid-holding conditions (non-irradiated sample).

After 48 h in liquid-holding, the p53-dependent apoptotic lymphocytes in the irradiated cultures presented shortened telomeres. After a 2-week recovery time, non-irradiated cells showed a p53-dependent spontaneous apoptosis, but no telomere shortening. These results demonstrate that radiation-induced apoptosis correlates with shortened telomeres in G₀ human lymphocytes. Spontaneous and radiation-induced apoptosis are dependent on expression of p53. In contrast, p53 may not play an effective role in telomere shortening, because spontaneous apoptosis did not correlate with telomere shortening. As most tumours are compromised with respect to p53 function, our findings on the role of p53 in telomere shortening may prove critical for applying therapeutic modalities in the clinic, and may facilitate the design of agents that selectively disrupt telomere integrity in tumour cells.

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1. Introduction

Apoptosis can develop spontaneously or be induced by exposure to agents such as ionizing radiation. It is well known that lymphocytes die spontaneously in culture. For cultures of rodent thymocytes, previous studies have indicated that spontaneous apoptosis affects 20–50% of the cell population within 16 h [1–3]. In addition, human lymphocytes are frequently used for studying the physiological impacts of radiation since immunocytes are relatively radiosensitive, readily accessible and relatively long-lived. Apoptosis of lymphocytes is a well-established and relatively specific biomarker for radiation-induced damage, and evasion of apoptosis is a hallmark of human cancers, such as hematological malignancies.

Genes involved in signalling DNA damage, such as p53, are important for apoptosis to occur [4]. Ionizing radiation is a potent inducer of DNA double-strand breaks (DSBs), and DSBs occurring

near telomeres would likely promote telomere loss [5]. Telomere shortening results in telomere dysfunction, a process also called telomere uncapping [6]. It is thought that the cell detects telomere shortening as DNA damage and will enter cellular senescence, growth arrest or apoptosis, depending on the cellular p53 status. However, the precise changes that occur at uncapped telomeres and the relationship between telomere uncapping and the cell cycle are not yet well understood.

Dicentric chromosomes can develop as a result of telomere shortening and fusion between the ends of chromosomes where the telomere structure is no longer able to suppress recombination. In fact, telomere shortening and chromosome fusion have been linked in a number of reports [7–9] and a significant correlation between dicentric and telomere shortening in the human lymphoblast cell-line WTK1 has been observed [10].

In our previous studies with G₀ human lymphocytes, a role of apoptosis in the selective removal of cells carrying dicentric chromosomes has been demonstrated [11,12]. In the present study, G₀ human lymphocytes from healthy donors were used to determine whether spontaneous or radiation-induced apoptosis correlates with telomere shortening and to study the role of p53.

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