NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

SBS Seminar Announcement

Using cloned mouse stromal cell lines as models to study mesenchymal stromal cell biology

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Abstract

My primary research interest has been the study of mouse lymphocyte development, focussing mostly on T cells. Model systems employed included the ontogeny of embryonic mouse thymus development in vivo and in vitro as well as the reconstitution of a thymus by donor progenitor cells in radiation bone marrow chimeras. The advent by Zuniga-Pflucker of culture systems employing OP9 stromal cells revolutionised our understanding of T cell development in vitro. The stromal cells used in these studies were developed by haematologists to support haematopoiesis and lymphopoiesis in vitro and represent what are called mesenchymal stromal cells. (MSC). Enigmatically, in addition to supporting haematopoiesis, MSC also inhibit the activities of mature lymphocytes making them potentially a useful tool with which to control immune responses. In the first part of my talk, some of our results characterising a collection of mouse stromal cell lines as well as freshly-isolated bone-marrow-derived cells will be presented.

The second half of my talk concerns the radiobiology of MSC. Following lethal total body irradiation, hematopoietic stem cells (HSC) do not survive, yet haematopoietic re-constitution by donor haematopoietic stem cells (HSCs) is supported by host-derived MSC that presumably survive irradiation. The mechanism(s) by which MSCs survive irradiation is poorly understood. The DNA Damage Response (DDR) represents a network of signalling pathways enabling cells to respond to genotoxic damage. We have previously shown that the execution of DDR pathways, including repair of DNA double strand breaks (DNA DSBs), promotes MSC survival post irradiation (IR)[1]. MSCs reside in a hypoxic (2-5% O_2) microenvironment in the bone marrow. Hypoxia is known to enhance the radio-resistance of cancer cells by altering their response to IR-induced DNA damage however, whether hypoxia affects the radio-resistance of MSCs is currently unknown.

We have studied the DDR of γ-irradiated mouse MSC lines, MS5 and ST2, cultured in normoxia (21% O_2) and hypoxia (5% O_2). Hypoxia increased MSC growth rate and enhanced long-term survival post irradiation. Cell cycle analysis by flow cytometry demonstrated that MSC recovery from IR (10 Gy) -induced cell cycle arrest was improved under hypoxic conditions. In MSCs, hypoxia accelerated the resolution of γ-H2AX expression (a marker of DNA DSBs) and the disappearance of γ-H2AX foci and induced increased expression of DNA DSB repair proteins, including DNA-PK_{cs} and DNA ligase IV in MSCs. MS5 and ST2 respond to hypoxia by up-regulating HIF-1¤ and HIF-2¤ transcripts and protein level, two key components controlling the response of cells to hypoxia.

Knock-down of HIF-1¤ resulted in increased IR sensitivity of MSC lines.
In summary, our results demonstrate, for the first time, that hypoxia enhances MSC radio-resistance *in vitro* most likely by the up-regulation of DNA DSB repair mechanisms, leading to alterations in the DDR to IR-induced DNA DSBs and thereby enhancing MSC survival. These results have important implications for our understanding of the role of MSCs in haematopoietic reconstitution as well as for their role in the tumour microenvironment.

Tuesday, 03 Sep 2013 2.30pm to 3.30pm SBS Classroom 4 (SBS-01n-24)

Host: Prof Klaus Erik Karjalainen