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Hypoxia Enhances the Radio-resistance of Mouse Mesenchymal Stromal Cells

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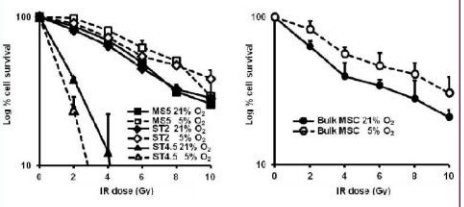
Abstract

Mesenchymal stromal cells (MSCs) are radio-resistant bone marrow progenitors that support hematopoiesis and its re-constitution following total body irradiation. MSCs reside in hypoxic niches within the bone marrow and tumor microenvironments. The DNA Damage Response (DDR) represents a network of signaling pathways that enable cells to activate biological responses to DNA damaging agents. Hypoxia-mediated alterations in the DDR contribute to the increased radio-resistance of hypoxic cancer cells, limiting therapeutic efficacy. The DDR is important in mediating mouse MSC radio-resistance. However, the effects of hypoxia on MSC radio-resistance are currently unknown.

In this study, we show how hypoxia increases long-term survival post irradiation and improves MSC recovery from IR-induced cell cycle arrest by the up-regulation of the DNA DSB repair machinery.

These findings have important implications for our understanding of MSC functions in supporting allogeneic bone marrow transplantation and in tumorigenesis. We are currently using these techniques to investigate the DDR of cortical and medullary thymic stromal cell lines.

1. Hypoxia enhances mouse MSC radio-resistance *in vitro*

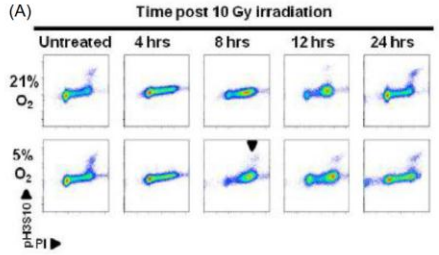


Clonogenic survival assays showed an increase in both mouse MSC cell lines (MS5 and ST2) and in bulk mouse MSC survival rates when these cells were cultured in hypoxia. MSC colony size was also increased in hypoxia under control and irradiated conditions.

In contrast to MSCs, in hypoxia, a $CD4^+/CD8^+$ lymphoma cell line (ST4.5) was more sensitive to irradiation and this sensitivity increased in a dose-dependent manner.

These results demonstrate that MSC radio-resistance was enhanced in hypoxia.

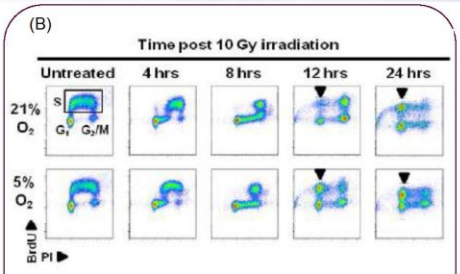
2. Hypoxia accelerates mouse MSC recovery from IR-induced cell cycle arrest



The G2/M checkpoint response of irradiated MSCs was studied by analyzing mitotic index using intracellular phosphorylated histone H3(Ser10) (pH3S10) staining by flow cytometry.

The absence of mitotic (pH3S10 positive) cells at early time-points (4-8 hrs) post IR confirmed that irradiated MS5 cells activated the G2/M checkpoint in both normoxia and hypoxia (A).

Recovery from G2/M arrest occurred earlier in hypoxia, indicated by the presence of mitotic MS5 cells at 8 hrs post IR in hypoxia (A, black arrowhead, lower 8 hr panel).



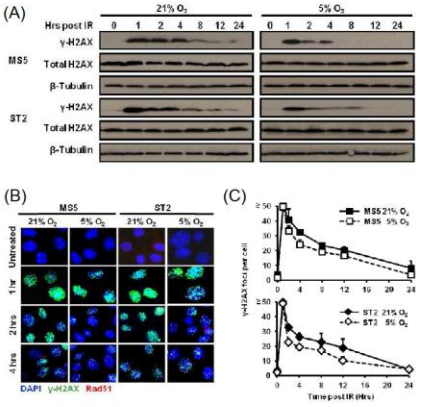
The intra-S-phase and G2 checkpoints were analyzed using a flow cytometry-based BrdU incorporation assay.

Increased proportions of BrdU labelled G1/S cells were present at 12 hrs and at 24 hrs post IR in hypoxia (B, black arrow heads).

These results indicated that MSC recovery from IR-induced cell cycle arrest was accelerated under hypoxic conditions.

3. DNA double-strand break repair is enhanced in mouse MSCs exposed to hypoxia

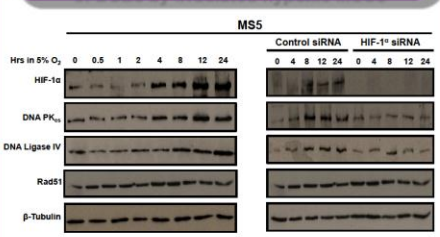
H2AX Ser139 phosphorylation is widely used as a marker for DNA double strand breaks (DSBs). This modification was studied by western blotting (A) and immunofluorescent staining (B and C).



H2AX Ser139 phosphorylation (A) and (ii) γ-H2AX IRIF (B and C) were resolved at a faster rate in irradiated MSCs cultured in hypoxia than in normoxia.

This suggests that hypoxia affects the DNA DSB repair capacity of mouse MSCs.

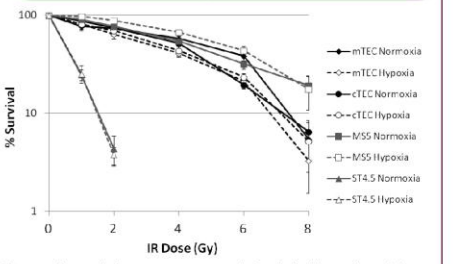
4. HIF-1α contributes to the resolution of DSBs by irradiated hypoxic MSCs



DNA ligase IV and DNA-PKcs (NHEJ repair proteins) expression levels correlated with the stabilization of HIF-1α in MSC hypoxic cells, while the levels of Rad51(involved in repair by HR) remained the same. This increase in the NHEJ-involved proteins was prevented when HIF-1α was knocked down.

This suggests that HIF-1α contributed to enhanced MSC radio-resistance in hypoxia by altering their DNA DSB repair capacity.

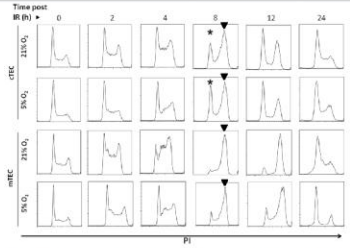
5. Medullary and Cortical Thymic Epithelial Cells show high radio-resistance



Clonogenic survival assays were used to study the radio-resistance showed by medullary and cortical thymic epithelial cells (mTEC and cTEC, respectively), using the MSC cell line MS5 and the lymphocyte cell line ST4.5 as radio-resistant and radio-sensitive controls.

Both mTECs and cTECs show a high radio-resistance, similar to that of MSCs. However, while the levels of oxygen do not affect cTEC survival rates, mTECs are slightly more radio-resistant when cultured in normoxia (21% O_2) than in hypoxia (5% O_2) , contrary to MSCs.

6. Medullary and cortical thymic epithelial cells Activate different checkpoints in response to IR



The different checkpoints activated in response to irradiation were studied by analyzing the PI profiles by flow cytometry.

While cTECs activate both G1 and G2/M checkpoints (*,▼), mTECs lack of G1 checkpoint activation and all the cells accumulate in G2 (▼). The cause for this lack of G1 checkpoint activation will be investigated in the future.

Future Work

1. Transcriptomic analyses of mouse MSCs to determine the transcriptional changes regulated by HIF-1α :
   - DNA Damage Response
   - DNA repair
   - Oxidative stress
2. Analyse the effects of therapeutics, e.g. HIF-1α inhibitors, on MSC radio-resistance.
3. Further characterize the DNA Damage Response of medullary and cortical thymic epithelial cells.

Conclusions

1. *Hypoxia increases MSC long-term survival post irradiation. It improves MSC recovery from IR-induced cell cycle arrest and accelerates the resolution of highly genotoxic IR-induced DNA double-strand breaks.*
2. *HIF-1α contributes to the resolution of DSBs, probably by up-regulating different proteins involved in DSB repair by NHEJ.*
3. *mTECs and cTECs are highly radio-resistant and activate different checkpoint in response to gamma irradiation.*

References

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