Effects of Hypoxia on the Radio-Resistance of Mouse Mesenchymal Stromal Cells and Thymic Epithelial Cells

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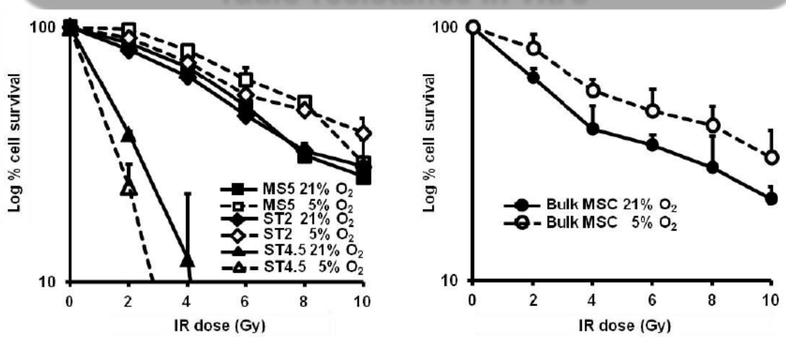
Abstract

Total body irradiation (TBI) is frequently used as a preparative regime prior to allogeneic BMT in order to eliminate endogenous HSCs and host immune cells to treat different diseases. Mesenchymal stromal cells (MSCs) are radio-resistant bone marrow progenitors that support hematopoiesis and its re-constitution following TBI. MSCs reside in hypoxic niches within the bone marrow, but they are found also in tumor microenvironments. We have shown that hypoxia is important in mediating mouse MSC radio-resistance by improving MSC proliferation rate, long-term survival, recovery from IR-induced cell cycle arrest and DNA DSB repair. HIF-1α contributes to this by increasing the expression of NHEJ DNA repair factors and regulating Rad51 foci formation in response to DNA DSBs. Our finding that hypoxia enhances mouse MSC radio-resistance in vitro has important implications for our understanding of MSC functions in supporting BMT and in tumorigenesis.

We are currently investigating the DDR of cortical and medullary thymic epithelial cell lines (mTEC and cTEC), which are essential for the development of functional T lymphocytes, so investigating the effects of irradiation on TECs is crucial for improving the outcomes of BMT. Our evidence suggests that hypoxia increases medullary TEC (mTEC) proliferation rates and colony sizes; however, mTECs show reduced survival rates under hypoxic conditions, which may be explained by the cells being more prone to undergo apoptosis under these conditions.

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

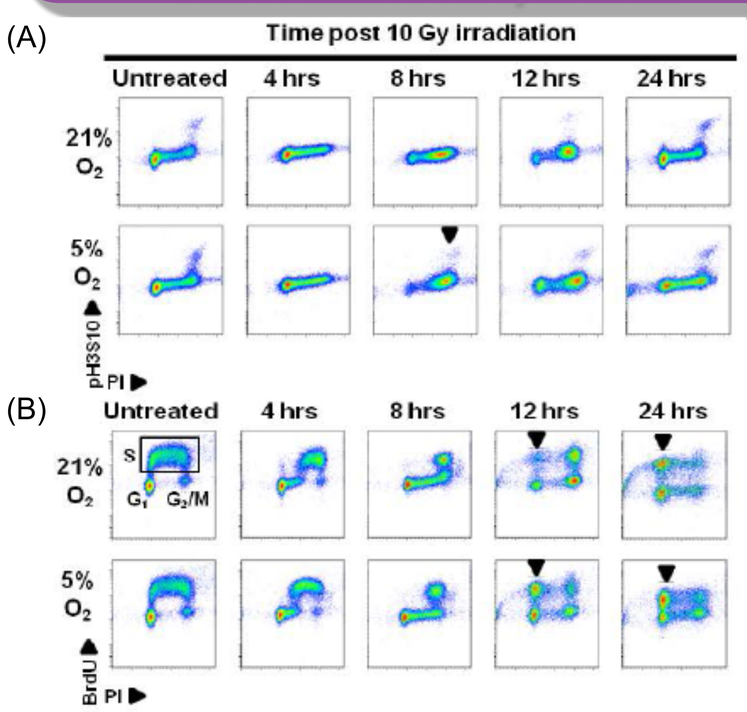


Clonogenic survival assays showed an increase in both mouse MSC cell lines (MS5 and ST2) and in bulk mouse MSC survival rates when these cells were cultured in hypoxia. MSC colony size was also increased in hypoxia under control and irradiated conditions.

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 (A), while the intra-S-phase and G2 checkpoints were analyzed using a flow cytometry-based BrdU incorporation assay (B).

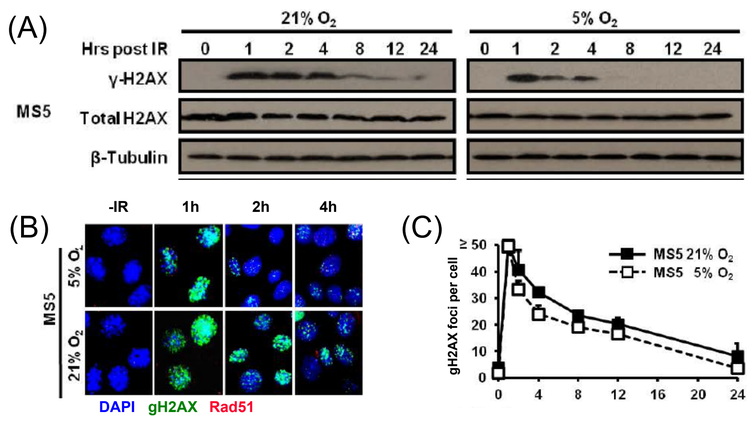
The absence of mitotic (pH3S10 positive) cells at early time-points (4-8 hrs) post IR confirmed that irradiated MS5 cells activated the G2/M checkpoint in both normoxia and hypoxia (A). Recovery from G2/M arrest occurred earlier in hypoxia, indicated by the presence of mitotic MS5 cells at 8 hrs post IR in hypoxia (A, black arrowhead, lower 8 hr panel).

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.

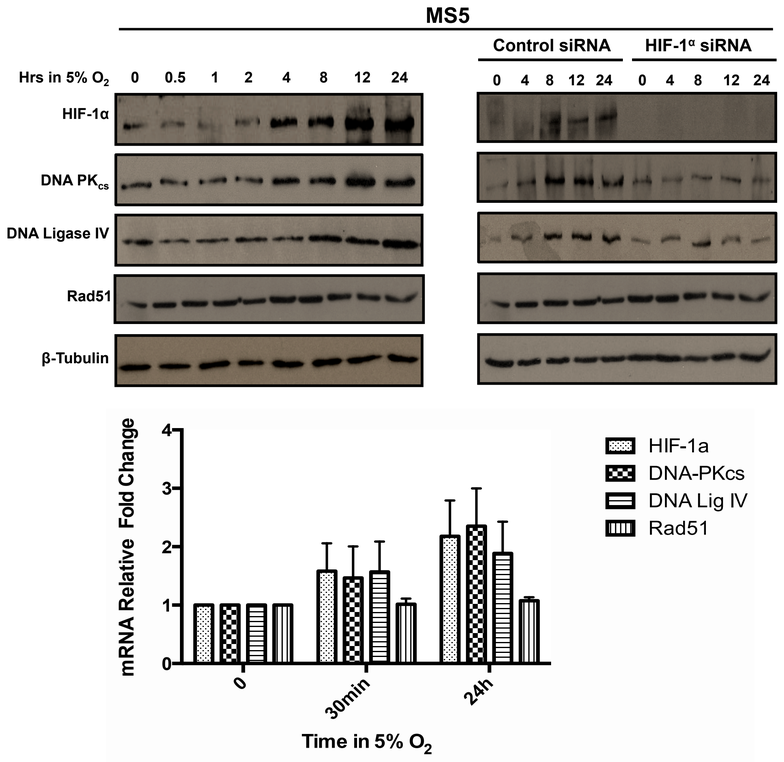
3. DNA double-strand break repair is enhanced in mouse MSCs exposed to hypoxia

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, **suggesting that hypoxia affects the DNA DSB repair capacity of mouse MSCs.**

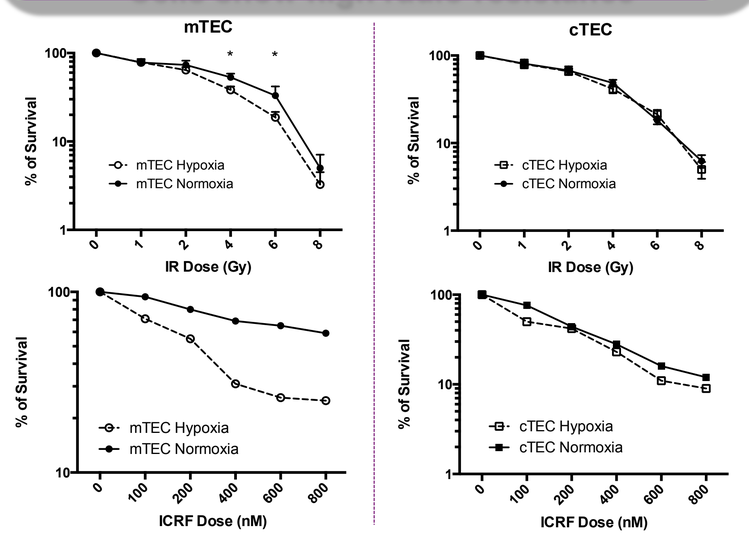
4. HIF-1α contributes to the resolution of DSBs by irradiated hypoxic MSCs



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.

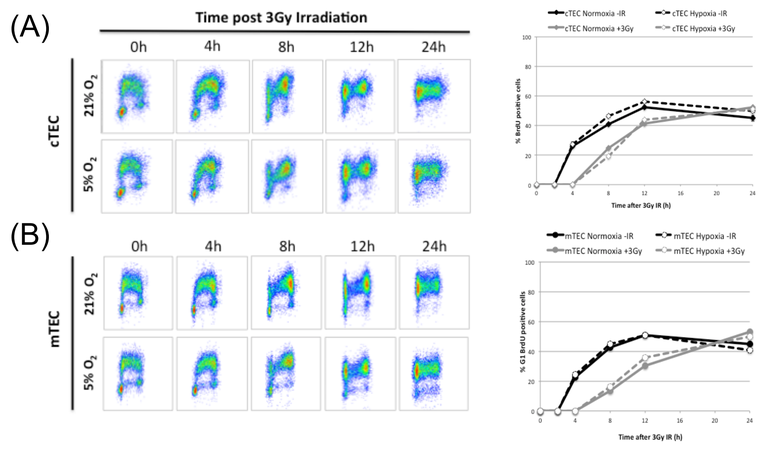
These results suggest that HIF-1α contributed to enhanced MSC radio-resistance in hypoxia by altering their DNA DSB repair capacity.

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). Both mTECs and cTECs show a high radio-resistance, similar to that of MSCs. However, **mTECs are slightly more radio-resistant when cultured in normoxia (21% O_2) than in hypoxia (5% O_2)**, contrary to MSCs. This is evidenced by both IR- and chemically- induced DNA damage. DNA repair capacity does not explain this result (data not shown).

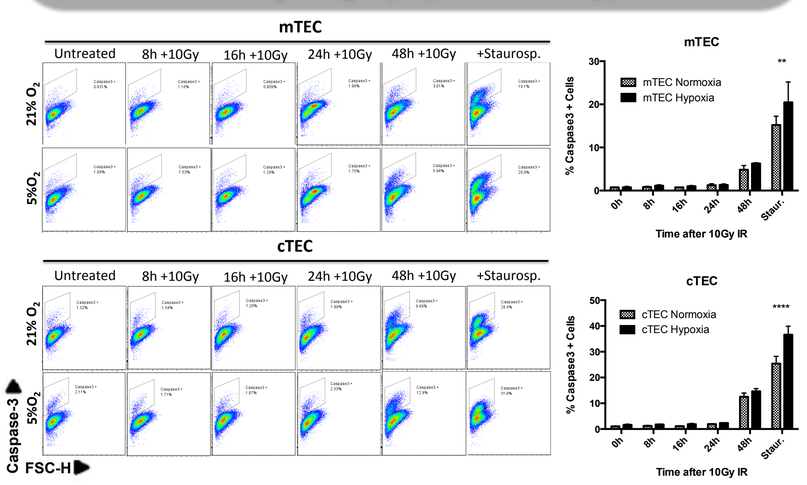
6. Oxygen level does not affect recovery from IR-induced cell cycle arrest on TECs



The different checkpoints activated in response to irradiation were studied by analyzing the BrdU/PI profiles of the irradiated cells compared to the unirradiated ones (A,B), while the G2/M checkpoint was analyzed by studying the mitotic index using the intracellular mitotic marker pH3S10, also by flow cytometry (data not shown).

Neither mTECs nor cTECs show any difference in the recovery from the IR-induced cell cycle arrest in Normoxia (21% O2) when compared to Hypoxia (5% O2), **suggesting that oxygen levels probably do not affect recovery from DNA Damage in TECs.**

7. Thymic Epithelial Cells may be more prone to undergoing apoptosis in Hypoxia



Apoptosis was addressed by measuring cleaved Caspase-3 positive cells by flow cytometry, using Staurosporin as a positive control. TECs (and mTECs in particular) seem to have higher apoptosis levels when cultured in Hypoxia compared to Normoxia, **suggesting that these cells may be more prone to undergo apoptosis under hypoxic conditions.**

Future Work

1. Transcriptomic analyses of mouse MSCs to determine the transcriptional changes in hypoxia, regulated and HIF-1α.
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 IR-induced DNA DSBs.*
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. mTEC survival to irradiation is enhanced in normoxic conditions, while that of cTECs is unnafected by O_2 levels. This cannot be explained by recovery after IR-induced cell cycle checkpoint activation or DNA repair capacity.*
4. *Hypoxia may enhance TEC susceptibility to undergo apoptosis following genotoxic insult.*

References

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Acknowledgements

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