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Regenerative Medicine Institute

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

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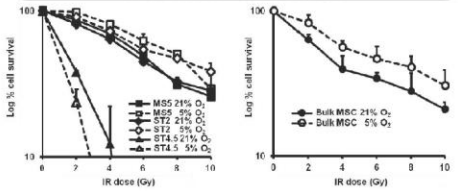
Abstract

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) is 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. In this case, hypoxia does not seem to have the same effects on the DDR of thymic epithelial cells than it has on that of MSCs.

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

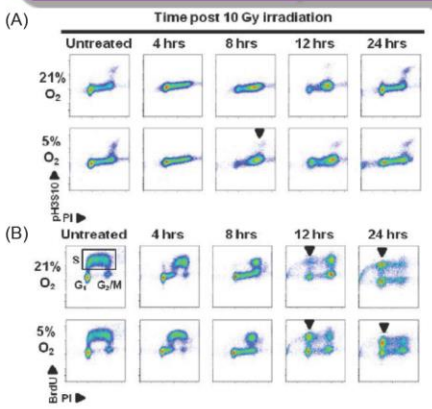


Clonogenic survival assays showed an increase in both mouse MSC cell lines (MS5 and ST2) and in bulk mouse MSC survival rates when these cells were cultured in hypoxia. MSC colony size was also increased in hypoxia under control and irradiated conditions.

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).

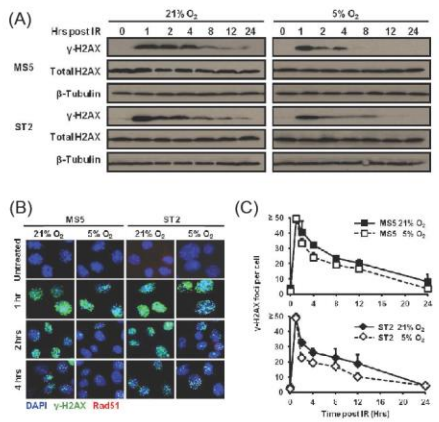
The absence of mitotic (pH3S10 positive) cells at early time-points (4-8 hrs) post IR confirmed that irradiated MS5 cells activated the G2/M checkpoint in both normoxia and hypoxia (A). Recovery from G2/M arrest occurred earlier in hypoxia, indicated by the presence of mitotic MS5 cells at 8 hrs post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

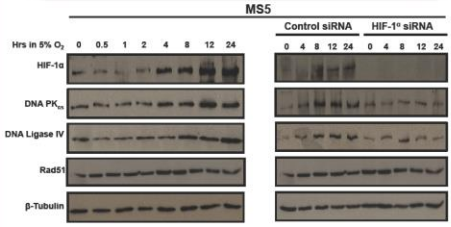
H2AX Ser139 phosphorylation is widely used as a marker for DNA double strand breaks (DSBs). This modification was studied by western blotting (A) and immunofluorescent staining (B and C).



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.

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

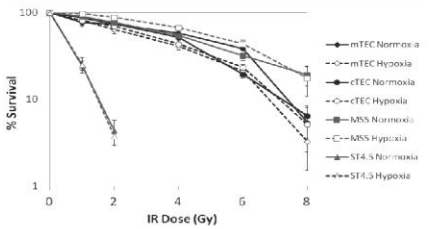
4. HIF-1α contributes to the resolution of DSBs by irradiated hypoxic MSCs



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.

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

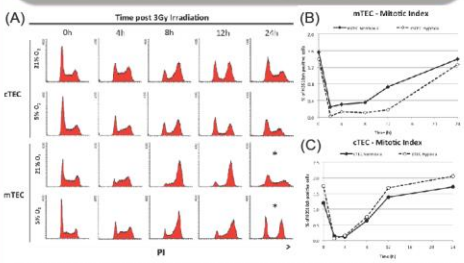
5. Medullary and Cortical Thymic Epithelial Cells show high radio-resistance



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

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.

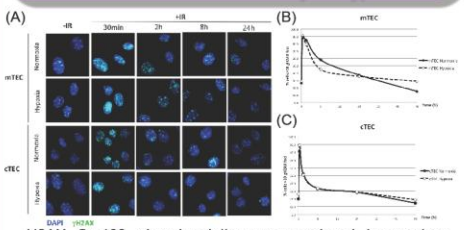
6. Medullary Thymic Epithelial Cells recover faster from IR-induced checkpoints in Normoxia



The different checkpoints activated in response to irradiation were studied by analyzing the PI profiles by flow cytometry (A) while the G2/M checkpoint response of irradiated TECs was studied by analyzing mitotic index using intracellular the mitotic marker pH3S10 by flow cytometry (B,C).

Preliminary results show that cTEC checkpoint activation and recovery does not seem to be affected by the oxygen levels (A,C). On the contrary, mTEC recovery from the IR-induced checkpoints seems to be delayed under hypoxic conditions, as shown by both PI staining and mitotic index analysis (A,B), which fits with the results from the radiation survival curves.

7. Medullary Thymic Epithelial Cells may have an enhanced DSB recovery in Hypoxia



H2AX Ser139 phosphorylation was analyzed by western blotting (data not shown) and γ-H2AX IRIF were studied by immunofluorescent staining (A).

Western blots show an accelerated phosphorylation of H2AX in Ser139 in irradiated mTECs exposed to hypoxia as well as a faster disappearence of this mark, which is commonly related with DSB repair (data not shown). Preliminary data obtained by Immunofluorescent staining of γ-H2AX IRIF also showed a faster DSB resolution in the case of mTECs (A,B), while there was no difference in cTECs.

Future Work

1. Transcriptomic analyses of mouse MSCs to determine the transcriptional changes regulated by 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 matches the data on recovery after IR-induced cell cycle checkpoint activation.*
4. *Hypoxia may enhance the DSB repair capacity of mTECs. However, further investigations are necessary in order to confirm it.*

References

Sugrue T, Lowndes NF & Ceredig Rh. (2014) *Stem Cells*, doi: 10.1002/stem.1683.
Sugrue T, Lowndes NF & Ceredig Rh. (2013) *Immunol. Cell Biol.* 91: 5-11.
Sugrue T, Brown JAL, Lowndes NF & Ceredig Rh. (2013) *Stem Cells* 31: 137-145..

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