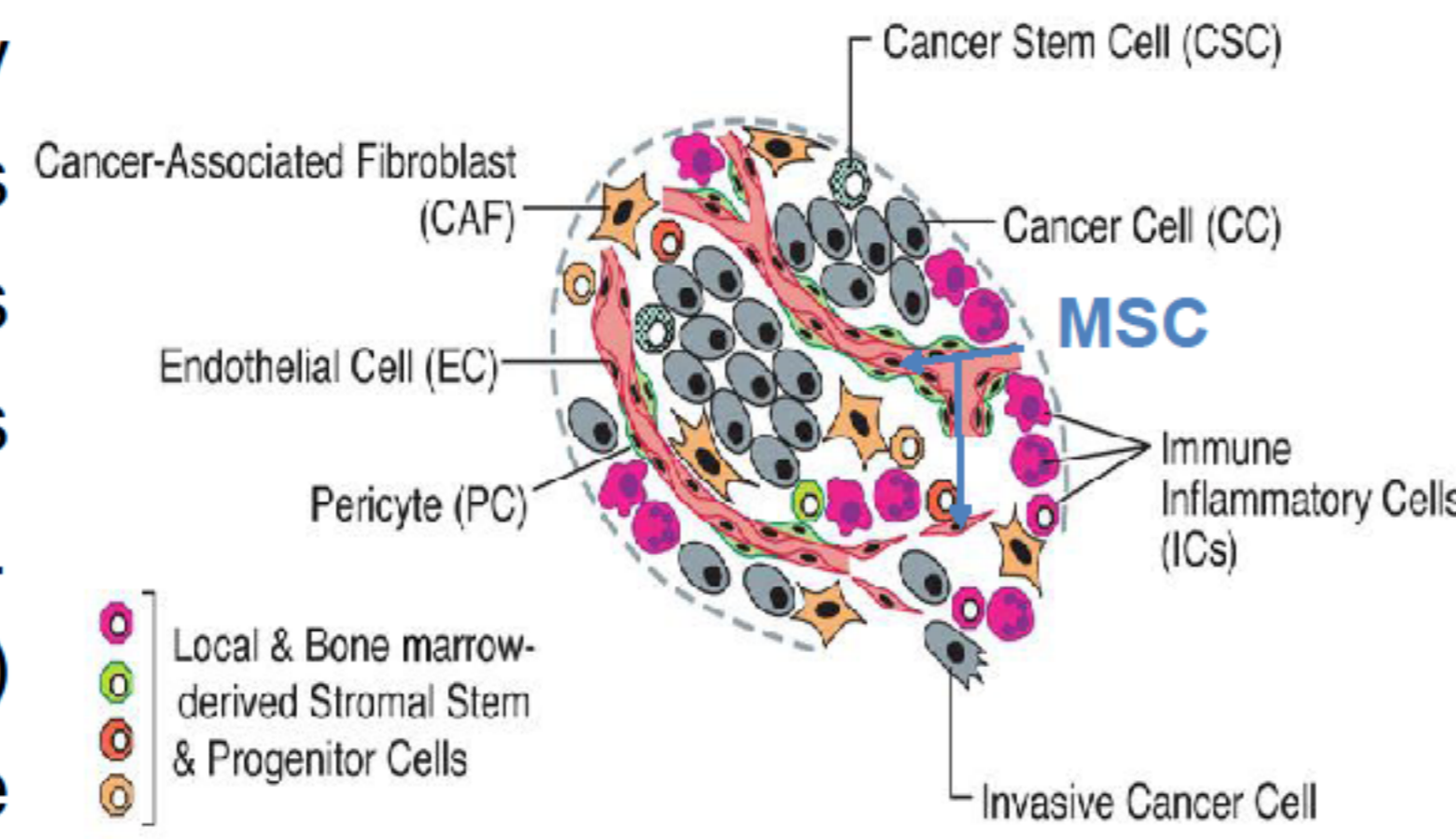




Introduction

The tumor-homing property of mesenchymal stem cells (MSC) has led to their use as delivery vehicles for therapeutic genes such as the sodium iodide symporter (NIS), which has convincingly been demonstrated by our recent studies of systemic NIS gene delivery using MSCs as delivery vehicles in both subcutaneous and orthotopic xenograft mouse models. External beam radiation therapy (EBRT) represents a promising tool in the application of engineered MSC (eMSC)-based gene therapy as tumor irradiation is likely to enhance MSC recruitment into irradiated tumor environments. This effect is presumably mediated through a radiation-induced stimulation of secretion of certain cytokines.



Hanahan D & Weinberg RA, Cell 2011; 144:646-74

Materials and Methods

Irradiation of human liver cancer cells (HuH7): HuH7 cells were irradiated with different doses (1-10 Gy) using the Xstrahl Cabinet Irradiator. Respective supernatants were removed and mRNA isolated at intervals from 0-48 h after irradiation.

Analysis of the effects of EBRT on the cytokine secretion profile

- 1) Expression levels of irradiated and non-irradiated HuH7 mRNA of factors associated with MSC recruitment were investigated by qPCR.
- 2) Furthermore, protein levels were quantified by ELISA using supernatants of irradiated and non-irradiated cells.

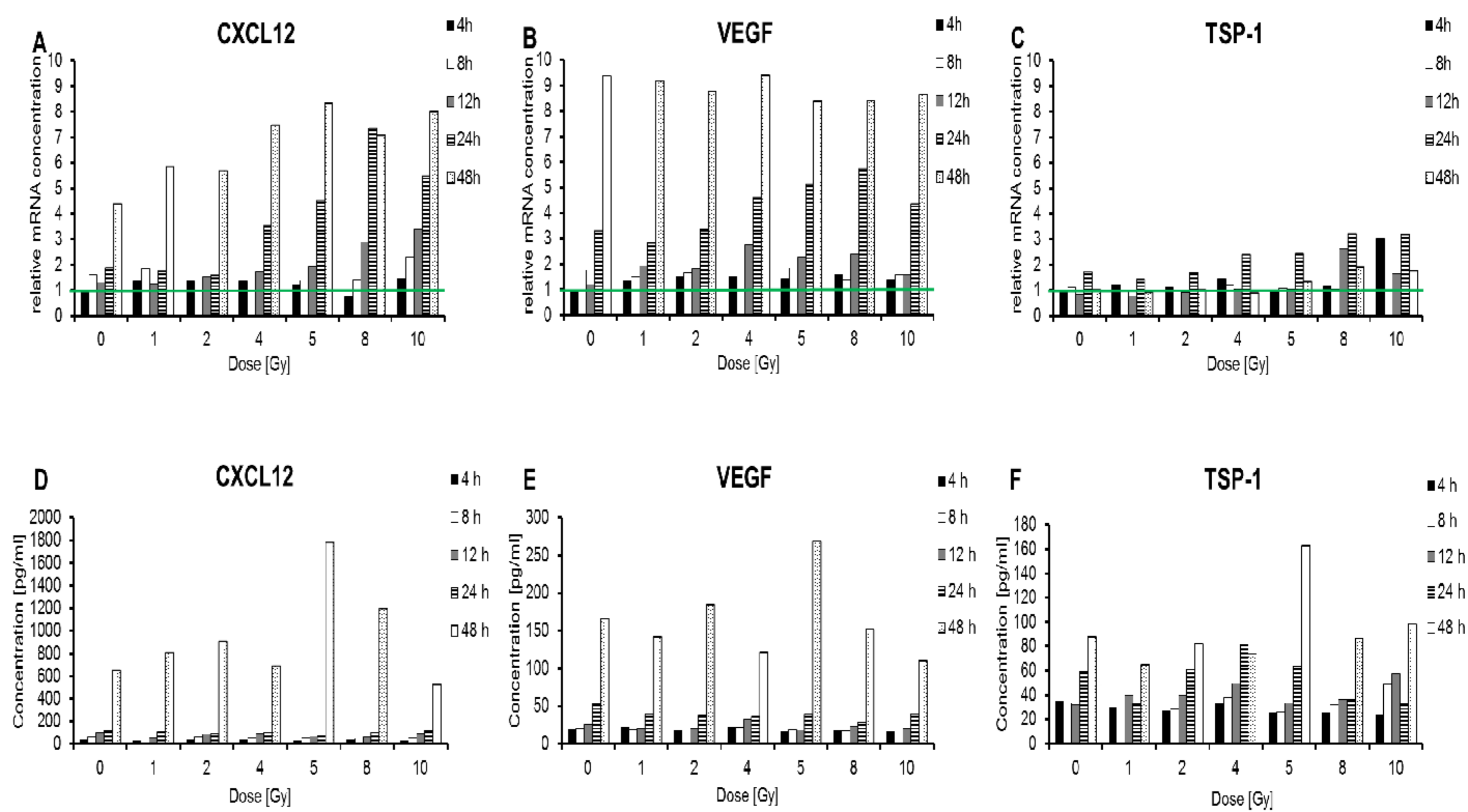
Chemotaxis assay: Chemotaxis of MSCs in relation to a gradient between irradiated and non-irradiated supernatants (48 h post irradiation) was further tested in a live cell tracking migration assay (IBIDI μ -slides Chemotaxis) and monitored by time-lapse microscopy for 24 h.

Results

Effects of EBRT on the cytokine secretion profile

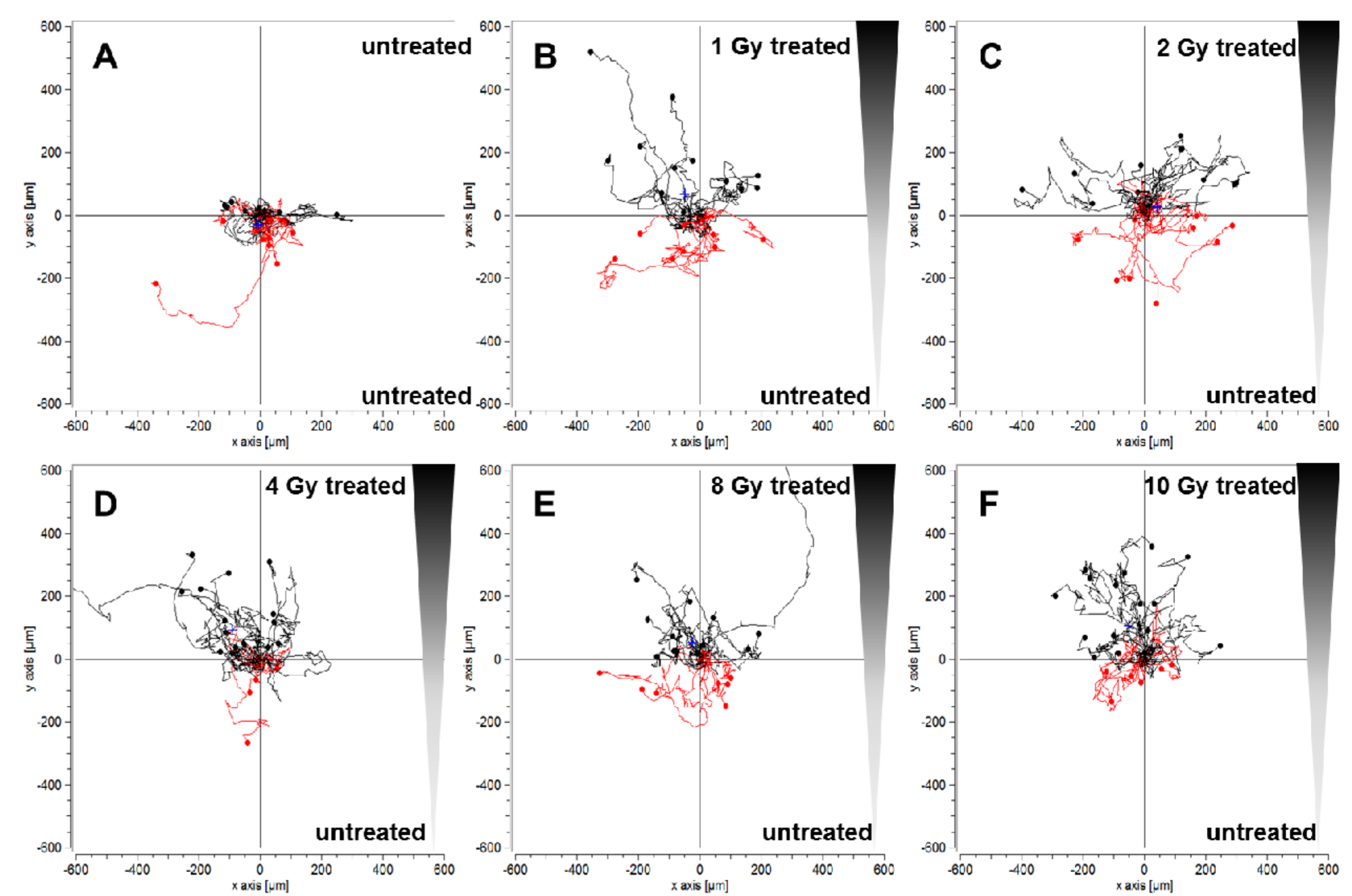
1) mRNA level: Compared to non-irradiated cells, irradiation led to a dose dependent increase of various cytokines and growth factors involved in MSC migration. For C-X-C motif chemokine ligand 12 (CXCL12), a radiation dose-dependent increase in mRNA concentration compared to non-irradiated cells was observed starting from 12 h post irradiation (A). This effect was stable up to 48 h after radiation treatment. An increase of up to 4-fold was measured 24 h after treatment with 8 Gy. Similar effects were seen for vascular endothelial growth factor (VEGF) (B) and thrombospondin-1 (TSP-1) (C), where a dose dependent increase in mRNA levels was seen after 12 and 24 h (up to 1.7-fold when treated with 8 Gy), and 24 and 48 h (1.5-fold after 24 h and 2-fold higher after 48 h at 8 Gy), respectively.

2) Protein level: Our findings were further confirmed by ELISA analysis. Beside an increase in VEGF (up to 1.6-fold) (E) and TSP-1 (up to 2-fold) (F) secretion, a high increase in CXCL12 expression (up to 3 fold) (D) was observed at 48 h post radiation.



Effects on migratory behavior of MSCs

MSCs subjected to supernatant from non-irradiated HuH7-cells in both chambers showed no directed chemotaxis (A). Cells under the influence of a gradient between non-irradiated and irradiated supernatants (1-10 Gy), showed directed chemotaxis (B-F). Quantification revealed a strong increase in mean forward migration index (yFMI) (G), mean center of mass (yCoM; blue crosses) (H) and mean directionality (I) of MSCs towards supernatants from irradiated compared to non-irradiated HuH7 cells signifying enhanced MSC migration.



Conclusions. Our data clearly demonstrate that tumor cells secrete higher levels of cytokines and growth factors that are involved in MSC tumor homing after irradiation resulting in stimulation of chemotaxis of MSCs towards tumor cells. This clearly shows the promising potential of EBRT pretreatment to enhance the migratory capacity of MSCs and thus tumor selectivity and therapeutic effectiveness of MSC-mediated gene therapy approaches.

Supported by:

