

Lasaviciute et al. Human bone-marrow mesenchymal stromal cell derived CXCL12, IL-6 and GDF-15 and their capacity to support IgG-secreting cells in culture are divergently affected by Doxorubicin.

SUPPLEMENTARY MATERIAL

Table S1. List of monoclonal antibodies used for flow cytometry.

CELL TYPE	ANTIBODY	FLUOROCHROME	DILUTION FACTOR	COMPANY
Human BM MSCs	CD73 (clone AD2)	V450	1:50	BD Biosciences
	HLA-ABC (clone DX17)	Alexa 488	1:20	BD Biosciences
	CD34 (clone 581)	PE-Cy7	1:40	BD Biosciences
	CD45 (clone 2D1)	APC-Cy7	1:40	BD Biosciences
	CD105 (clone 43A3)	PE	1:50	Biolegend
MBCs and ASCs	CD20 (clone LT20)	PerCP	1:20	Miltenyi Biotec
	CD27 (clone M-T271)	V450	1:40	BD Biosciences
	CD38 (clone HIT2)	FITC	1:10	BD Biosciences
	CD138 (clone MI15)	APC	1:40	Biolegend

BM MSCs: bone-marrow mesenchymal stromal cells, MBCs: memory B cells, ASCs: antibody-secreting cells

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Table S2. Soluble proteins derived from *in vitro* cultures of Doxo-exposed and non-exposed human bone-marrow derived mesenchymal stromal cells.

Analyte	Spot density											
	Donor 1 ALL		Donor 2 ALL		Donor 3 ALL		Donor 4 HC		Donor 5 HC		Donor 6 HC	
	0.0 µg/ml Doxo	0.5 µg/ml Doxo	0.0 µg/ml Doxo	0.5 µg/ml Doxo	0.0 µg/ml Doxo	0.5 µg/ml Doxo	0.0 µg/ml Doxo	0.5 µg/ml Doxo	0.0 µg/ml Doxo	0.5 µg/ml Doxo	0.0 µg/ml Doxo	0.5 µg/ml Doxo
Adiponectin	0	0	0	0	0	1	0	0	1	0	1	0
Apolipoprotein A-I	0	0	0	0	0	0	0	0	0	0	1	0
Angiogenin	6	6	6	5	3	1	6	6	7	6	2	5
Angiopoietin-1	1	1	1	0	2	1	5	5	5	2	1	3
Angiopoietin-2	4	3	4	3	2	1	4	3	5	2	1	1
BAFF	0	0	0	0	1	0	0	0	0	1	0	0
BDNF	4	5	3	4	1	3	3	5	3	2	0	2
Complement component C5/5A	0	0	0	0	1	0	0	0	0	0	0	0
CD14	0	0	0	0	0	0	1	1	0	0	0	0
CD30	0	0	0	0	0	0	0	2	0	0	0	0
CD40 ligand	0	0	0	0	2	2	0	3	2	1	1	1
Chitinase3-like	5	1	7	0	1	5	9	8	9	9	1	5
Complement Factor D	0	0	2	0	1	1	2	2	3	2	0	0
C-Reactive Protein	0	0	0	0	0	1	1	2	1	1	1	0
Cripto-1	0	0	0	0	0	1	-	0	0	0	1	0
Cystatin C	0	0	1	1	0	1	2	3	1	2	1	1
Dkk-1	9	8	9	9	8	9	10	9	9	9	6	9
DPPIV	0	0	0	0	0	0	0	0	0	0	0	0
EGF	0	0	0	0	0	0	0	0	0	0	0	0
EMMPRIN	2	1	2	0	2	2	4	3	3	3	1	3
ENA-78	0	0	0	0	3	1	1	2	0	1	0	1
Endoglin	5	2	5	2	2	1	6	5	6	5	1	4
FasLigand	0	0	0	0	0	0	0	1	0	0	0	0
FGFbasic	0	0	1	0	1	3	2	3	2	1	1	1
FGF-7	0	0	0	0	0	0	2	1	1	0	0	1
FGF-19	5	5	5	5	5	5	5	6	5	5	5	5
Flt-3Ligand	0	1	1	1	0	0	0	2	1	1	0	1
G-CSF	0	0	0	0	0	0	0	1	0	0	0	0
GDF-15	2	7	0	8	2	5	6	8	5	8	0	6
GM-CSF	1	1	2	0	1	2	2	2	0	1	0	1
GRO-α	0	0	0	0	0	0	0	1	0	0	0	0
GrowthHormone	0	0	0	0	0	0	0	0	0	0	0	0
HGF	0	0	1	0	0	0	1	1	1	0	0	0
ICAM-1	0	0	0	0	0	0	1	1	1	0	0	0
IFN-γ	0	0	0	0	0	1	1	1	1	1	1	1
IGFBP-2	6	7	4	6	0	1	2	5	0	3	0	5
IGFBP-3	6	5	6	5	4	2	5	6	6	5	1	4
IL-1α	0	0	1	0	0	0	0	0	0	0	0	0
IL-1β	0	0	0	0	1	0	0	1	0	0	0	0
IL-1ra	0	0	2	0	1	0	0	1	0	0	0	1
IL-2	0	0	0	0	0	0	0	1	0	5	0	0
IL-3	0	0	0	0	0	0	1	1	0	0	0	0
IL-4	2	0	0	1	1	1	2	3	1	1	1	1
IL-5	0	0	0	0	0	0	0	0	0	0	0	0
IL-6	4	6	5	7	5	6	5	6	6	7	4	6
IL-8	7	7	6	8	6	7	10	9	8	7	5	7
IL-10	0	0	0	1	1	1	3	2	1	1	1	1
IL-11	4	2	4	4	5	4	5	5	3	2	2	5
IL-12p70	0	0	0	0	1	0	0	1	0	0	1	1
IL-13	0	0	0	0	0	0	0	1	0	0	0	0
IL-15	0	0	0	0	0	0	0	1	0	0	0	0
IL-16	0	0	0	0	0	0	0	1	0	0	0	0
IL-17A	5	5	5	5	6	5	6	6	5	5	5	5
IL-18BPα	0	0	0	0	0	0	0	0	0	0	0	0

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IL-19	0	0	0	0	0	0	0	0	0	0	0	0
IL-22	3	1	1	1	1	2	2	2	1	2	1	3
IL-23	0	0	0	0	0	0	0	0	0	0	0	0
IL-24	0	0	0	0	0	0	1	0	0	0	0	0
IL-27	0	0	0	1	0	1	2	1	0	0	0	1
IL-31	0	0	0	0	0	0	0	0	0	0	0	0
IL-32 abg	0	0	0	0	0	0	0	1	0	0	0	5
IL-33	0	0	0	0	0	0	0	1	0	0	0	0
IL-34	0	0	0	0	0	0	0	1	0	0	0	0
IP-10	0	0	0	0	0	0	0	1	0	0	0	0
I-TAC	0	0	0	0	0	1	0	1	0	0	0	0
Kallikrein3	0	0	0	0	0	1	0	1	0	0	0	0
Leptin	0	0	0	0	0	0	0	0	0	0	0	0
LIF	0	0	0	1	0	0	1	0	1	1	0	3
Lipocalin-2	0	0	0	0	1	0	1	0	0	0	0	0
MCP-1	7	6	7	8	8	7	8	7	8	7	6	8
MCP-3	0	0	0	0	0	1	1	1	0	1	1	0
M-CSF	0	0	0	0	0	1	1	1	0	0	1	0
MIF	4	2	3	4	3	2	5	5	5	5	1	5
MIG	0	0	0	0	0	1	0	1	0	0	0	0
MIP-1 α /MIP-1 β 6348/6351	0	0	0	0	0	1	0	0	0	0	0	0
MIP-3 α	0	0	0	0	0	1	0	0	0	0	0	0
MIP-3 β	0	0	0	0	0	1	0	1	0	0	0	0
MMP-9	0	0	0	0	0	1	0	1	0	0	0	0
Myeloperoxidase	0	0	0	0	0	0	0	0	0	0	0	0
Osteopontin	4	3	4	5	5	4	3	3	3	3	4	5
PDGF-AA	1	0	0	1	3	1		2	1	2	0	2
PDGF-AB/BB	0	0	0	0	0	0	0	0	0	0	0	0
Pentraxin-3	4	4	4	5	3	2	4	3	6	5	2	4
PF4	0	0	0	0	0	0	0	0	0	0	0	0
RAGE	0	0	0	0	0	1	0	1	0	0	0	0
RANTES	0	0	0	0	0	1	0	1	0	0	0	0
RBP4	0	0	0	0	1	1	0	1	0	0	0	1
Relaxin-2	0	0	0	0	1	1	0	1	0	0	0	1
Resistin	1	0	0	0	2	2	1	2	0	1	0	5
SDF-1 α	6	5	6	4	5	3	6	5	6	5	5	4
SerpinE1	10	10	10	10	10	10	10	10	10	10	10	10
SHBG	0	0	0	0	0	1	2	0	2	3	1	2
ST2	0	0	0	0	0	0	1	0	0	0	0	1
TARC	0	0	0	0	0	1	1	0	0	0	0	1
TFF3	0	0	0	0	0	0	0	0	0	0	0	0
TfR	0	0	0	0	0	0	1	0	0	0	0	1
TGF- α	0	0	0	0	0	0	0	0	0	0	0	0
Thrombospondin-1	7	7	7	8	7	5	8	7	7	7	6	8
TNF-alpha	0	0	0	0	0	1	0	0	0	0	1	0
uPAR	6	5	6	6	0	1	5	6	6	6	1	5
VEGF	5	4	6	5	3	1	5	5	6	5	1	4
VitaminD BP	0	0	0	0	0	0	0	0	0	0	0	0

Data derived from n=6 unique donors: n=3 patients under maintenance therapy against paediatric acute lymphoblastic leukaemia, and n=3 healthy controls (HC). Values depict dot intensity ranging from either absence (0) to maximum expression (10).

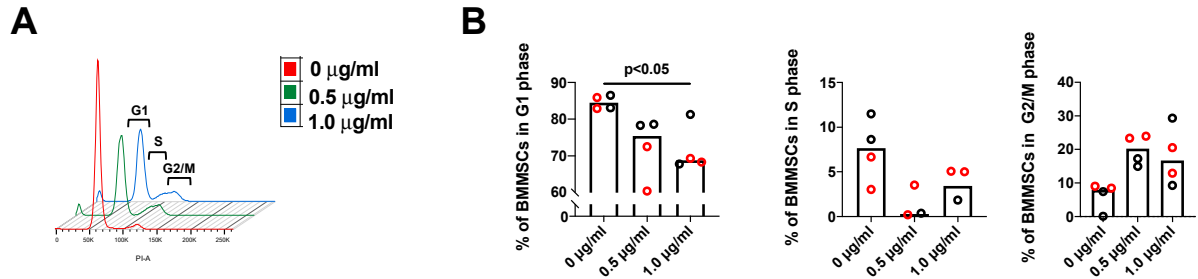
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Table S3. Oligonucleotides used as qPCR primers.

Gene	Forward	Reverse
CXCL12	GTGGTCGTGCTGGTCCTC	GCATGGGCATCTGTAGCTC
IL-6	AAAGAGGCACTGGCAGAAAA	TTTCACCAGGCAAGTCTCCT
GDF-15	GAGCTGGGAAGATTCGAACA	AGAGATACGCAGGTGCAGGT
APRIL	GACTTTCACCATGGGTCAGG	TCCCCTTGGTGTAAATGGAA
BAFF	CCATGTCTTTGGGGATGAAT	GGAGTTCATCTCCTTCTTCCAG
Reference gene	Forward	Reverse
TFRC	TCTGGAATCCCAGCAGTTTC	CGTGCCACTTTGTTCAACTC

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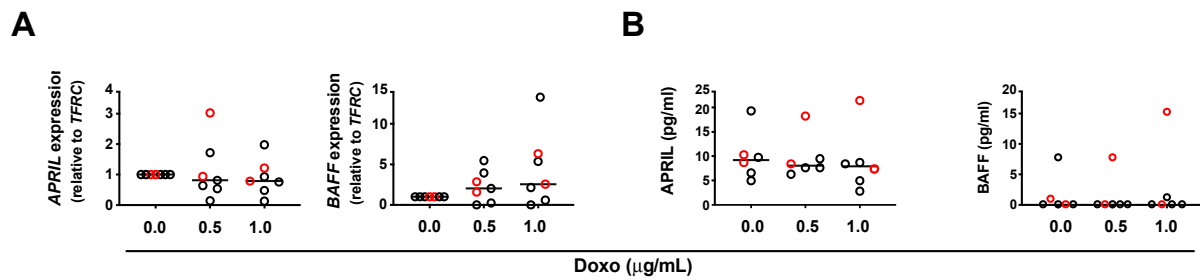
Figure S1 Cell cycle analysis following *in vitro* exposure of bone-marrow mesenchymal stromal cells to Doxorubicin.



(A) Representative and **(B)** compiled data of cell cycle analysis showing the percentage of propidium iodide-stained BM MSCs upon exposure to different doses (µg/mL) of Doxorubicin (n=4 unique donors). All data were acquired during one independent experiment. **●**: Healthy controls, **●**: Paediatric subjects under treatment for acute lymphoblastic leukaemia.

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Figure S2. Bone-marrow mesenchymal stromal cells produce minor levels of APRIL and BAFF regardless of Doxorubicin exposure.



(A) Gene expression (n=7 unique donors) and **(B)** secretion of APRIL and BAFF (n=6 unique donors), measured in BM MSCs by qPCR and ELISA respectively, following 0.0, 0.5 or 1.0 $\mu\text{g/mL}$ Doxo exposure. The mRNA levels were normalized to *TFRC* reference gene and Doxo treated groups were compared assigning the arbitrary value 1 to gene expression in the non-Doxo treated samples (0.0 $\mu\text{g/mL}$). All data were acquired during one independent experiment. Bars represent median. **O**: Healthy controls, **O**: Paediatric subjects under treatment for acute lymphoblastic leukaemia.