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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Mitochondrial dsRNA from B-Precursor Acute Lymphoblastic Leukemia Cells Induce Formation of Cancer Associated Fibroblast in the Bone Marrow Leading to Formation of a Chemoprotective Bone Marrow Niche

Aditi Dey[1], Richard Burt, PhDFRCPath,MRCP[2,3,4], Emily Annie Cutler, MSc[1], Hermione Elsie Allen, MSc[1], Jenny Chatzigerou, MSc[1], Rodothea Amerikanou, MBBChir[1], Samantha Atkinson, BSc[3,4], Ashish Dhir, PhD[5], Kristina Kirschner, PhD[6], Adele Kay Fielding, MBBS,PhD[7,8]

[1] University College London, London, United Kingdom
[2] University College London Hospital, London, United Kingdom
[3] The Francis Crick Institute, London, United Kingdom
[4] Imperial College London, London, United Kingdom
[5] University of Edinburgh, Edinburgh, United Kingdom
[6] Cancer Research UK Beatson Institute, Glasgow, United Kingdom
[7] UCL Cancer Institute, London, United Kingdom
[8] Centre for Blood Research, University of York, York, United Kingdom

Precursor B-cell Acute Lymphoblastic Leukemia (B-ALL) is typically a highly chemosensitive malignancy with the majority of children being cured by chemotherapy alone. Adult ALL is often chemosensitive at initial treatment, but long term disease free survival is less common. It is widely accepted that outcome in ALL closely relates to underlying genetic lesion (Moorman, Hematologica 2016) but no specific mechanisms by which ALL genetics influence outcome have been identified.

We have previously demonstrated that an 'activated mesenchymal stromal cell (MSC) niche' develops in response to reactive oxygen species (ROS) -inducing chemotherapy in which mitochondria are actively transferred along tunnelling nanotubes to 'rescue' B-ALL cells from chemotherapy (Burt, et al Blood 2019). According to recent evidence that chemotherapy resistant ALL sub-clones may exist at diagnosis (Dobson et al Cancer Discovery 2020), we hypothesised that B-ALL cells may themselves directly activate bone marrow stromal cells to generate cancer associated fibroblasts (CAF) from MSC.

To test this hypothesis, we first used our previously described *in vitro* niche model wherein either the HS27a MSC cell line or healthy donor (HD) MSCs are co-cultured with either B-ALL cell lines or primary patient B-ALL cells. Three of five B-ALL cell lines and 3 of 4 primary B-ALL samples tested, induced activation of the MSCs, as characterised by cytomorphological changes identified by confocal microscopy, up-regulation of CAF-relevant gene expression and increased IL6, IL8 and CCL2 protein secretion. The ability of the B-ALL cell lines to induce CAF correlated directly with the intrinsic ROS levels of the cell as determined by CellROX assay. *In vivo*, the CAF-inducing ALL cell lines also activated murine MSCs when established as xenografts in NOD-SCID gamma (NSG) mice. Immunohistochemistry of NSG murine femora revealed a very significant expansion in nestin+ cells after exposure to CAF-induing ALL cells compared to non-CAF-inducing controls. We established that the ALL cells activated MSC without contact, both from exposure of MSC to ALL cells in transwell and from exposure to B-ALL cell conditioned media (CM).

RNA sequencing of HS27a stromal cells exposed to CAF-inducing B-ALL cell CM compared with non-CAF inducing controls unexpectedly showed that the most highly upregulated pathways were those involved in RNA sensing. Using a smaller gene panel selected from among the RNA-sensing pathway genes, we confirmed that this was also the case for primary, HD MSCs upon exposure to primary ALL patient samples.

As a result, we sought evidence for the role of mitochondrial dsRNA, which has been shown to activate the anti-viral signalling pathway (Dhir et al, Nature 2018).

Using the J2 anti-dsRNA monoclonal antibody in both confocal and flow cytometric assays, we observed very significantly higher levels of dsRNA in MSC which had been exposed to CAF inducing B-ALL cell CM than in non-CAF inducing B-ALL or MSC alone controls. We confirmed by RT-PCR for mitochondrial genes on dsRNA immunoprecipitated from CAF inducing B-ALL cell CM that the dsRNA was of mitochondrial origin. Using ELISA, we quantified dsRNA from total cell RNA extracted from paired primary B-ALL patient samples at diagnosis and in remission. We found significantly higher levels of dsRNA at

diagnosis than in remission, with it being undetectable in most remission samples. Moreover, we confirmed by flow cytometry the presence of dsRNA in 6 B-ALL patient derived xenografts which was absent in the control NSG mice without leukemia.
We used DMSO to degrade dsRNA from CAF inducing B-ALL cell CM, as is often done in Sanger sequencing and confirmed the degradation by ELISA. We then quantified gene expression of a selection of stromal cell activation marker genes, as well as genes in the RNA sensing pathway after exposure to either DMSO-treated B-ALL cell CM or non-treated control. There was a 2 to 4-fold reduction in expression of IL6, IL8 as well as of RIG-I, MX2, IFIH1, MAVS and OAS1 expression in DMSO-treated condition compared to control. We confirmed that IL6, IL8 and CCL2 protein secretion was also significantly abrogated in the test but not the control condition.
We propose a model in which ROS-induced mitochondrial dysfunction in ALL cells leads to release of dsRNA which in turn activates bone marrow stromal cells leading to protective niche formation, despite lack of prior exposure to cytotoxic agents.

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