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Title: M2-LIKE MONOCYTE-DERIVED MACROPHAGES PROTECT AML CELL LINES AND PRIMARY AML CELLS AGAINST THERAPY-INDUCED APOPTOSIS

Abstract Type: Poster Presentation

Session Title: Hematopoiesis, stem cells and microenvironment

Background:

In the bone marrow (BM) of acute myeloid leukaemia (AML) patients, leukaemic cells co-exist with various BM-resident cells and infiltrating immune cells (e.g., monocytes/macrophages). Macrophages (Mφs) are highly plastic cells and exist on a functional polarization spectrum, broadly classed as either M1-like (pro-inflammatory, anti-tumourigenic, CD86⁺) or M2-like (anti-inflammatory, pro-tumourigenic, CD163⁺CD206⁺). Importantly, AML patient BM contains a higher number of CD163⁺CD206⁺ M2-like Mφs, compared to the BM of healthy individuals, that correlate with reduced response to induction therapy. Despite these findings, the direct capacity of M2-like Mφs to drive therapy resistance in AML and the molecular mechanisms involved have not been fully elucidated to date.

Aims:

Our aim was to investigate the ability of M2-like monocyte-derived Mφs (MDMs) to protect AML cell lines and primary cells against therapy-induced apoptosis and uncover the potential mechanisms underlying this resistance.

Methods:

Healthy BM and AML BM samples were immunophenotyped using multiparameter flow cytometry, to ascertain and compare the levels of M1-like (CD34⁻CD117⁻CD14⁺CD86⁺) and M2-like (CD34⁻CD117⁻CD14⁺CD163⁺CD206⁺) Mφs. Healthy donor CD14⁺ monocytes were isolated from peripheral blood and differentiated into M2-like Mφs with M-CSF for 10 days, with a media change included at day 7. On day 10, the media was collected, filtered and utilised as MDM conditioned media (CM), enriched in factors secreted by the MDMs. AML cell lines (U937, THP-1, KG-1a) and primary AML cells, obtained via leukapheresis, were cultured alone (monoculture), in the presence of 50% MDM CM or in direct co-culture with MDMs and treated with daunorubicin (DNR) or cytarabine (Ara-C) for 24 (U937, primary AML), 48 (KG-1a) or 72 hours (THP-1). Apoptosis was assessed by eFluor450/Annexin V staining and flow cytometry.

Results:

Immunophenotypic analysis revealed that AML BM exhibits a higher percentage of M2-like Mφs (72.6%) compared to a healthy BM (9.4%) that predominantly contains CD86⁺ M1-like Mφs (69.5% in normal BM versus 48.3% in AML BM), confirming previous findings. Direct co-culture with primary MDMs protected AML cell lines and primary AML patient samples against therapy-induced apoptosis. Indeed, MDMs completely abrogated the killing effects of DNR and Ara-C in AML cells, recovering the viable cell counts to near baseline levels. Intriguingly, 50% MDM CM also successfully promoted AML cell viability in KG-1a cells in the presence of DNR and in primary cells during culture with low-dose DNR and Ara-C. Moreover, 50% MDM CM protected against spontaneous apoptosis in an AML patient sample.

Summary/Conclusion:

These data reveal, for the first time, the ability of M2-like MDMs to protect AML cell lines and primary AML samples against therapy-induced apoptosis. Further studies are required to understand the mechanism(s) by which MDMs provide this protection, and these studies are currently ongoing in our laboratory. We propose that the observed protective phenotype is acquired via a combined mechanism involving direct Mφ-to-AML cell contact and Mφ secreted factors.

Keywords: Bone marrow microenvironment, Macrophage, Drug resistance, Acute myeloid leukemia