Variation in Extracellular Vesicles Among Clonally Derived Mesenchymal Stromal Cell Populations

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INTRODUCTION: The clinical efficacy of culture expanded polyclonal human mesenchymal stromal cell preparations (hMSCs) in Phase II trials for tissue regeneration and immunomodulation has been limited. The field has recently focused on the challenge of batch-to-batch variation of MSCs in both biological effects and secretome, including extracellular vesicles (MSC EVs), in an attempt to isolate and improve desirable MSC-derived efficacy. Our laboratory has been working to understand the source of batch-to-batch MSC variation and improve MSC manufacturing precision by generating clonal MSC populations from single colony founding connective tissue progenitors (CTPs) and examining clonal variation in biological performance and in secretome, including extracellular vesicles (EVs). We hypothesize that competitive expansion of heterogeneous CTP-derived clones results in stochastic variation in outcome of MSC manufacturing, which can be limited through a process of rational clone selection or removal. A foundation on which to begin choosing desirable and eliminating undesirable clones requires an understanding of the variation in performance between clones and the attributes of CTPs and CTP-derived clones that predict desirable performance.

METHODS: Human cells from a single donor were isolated from femoral metaphyseal bone discarded at the time of hip arthroplasty and cultured at clonal density in αMEM and 10% selected FBS lot for 8 days with daily phase contrast imaging to identify clonal colonies derived from a single founding cell (CTP). 4 clonal colonies were individually cultured to passage 5. MSC EVs and non-EV secretomes were isolated from the cells after 48 hours in serum free media by ultracentrifugation and banked at -80°C (consistent with MISEV 2018 guidelines). EV purity and morphology was confirmed by electron microscopy (Fig 1). 48 biochemical analytes were measured via Luminex multiplex on lysed/non-lysed EVs and residual non-EV secretome from each MSC clone population. This study was IRB approved.

RESULTS SECTION: Each clone demonstrated large differences in analyte levels in EV and non-EV secretome fractions (Table 1). Neuropilin-1 and IFN-α were elevated in lysed EVs. IL-1ra was elevated in whole EVs. IL-6, IL-8, MMP-1, MMP-2, and MCP-1 were elevated in non-EV secretome fractions.

DISCUSSION: Culture expanded clonal populations of hMSCs can be generated. These populations differ quantitatively in biological performance, morphology, and in secretome profile in both EV and non-EV components. These differences may be used to parse the variation between CTP-derived MSC populations and make more informed decisions regarding clone selection for MSC manufacture, and improve consistency in therapeutic potency. The high prevalence of Neuropilin-1 in MSC-derived EVs is of particular relevance given the protein's interaction with VEGF-A in settings of angiogenesis.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding clonal differences in CTP-derived culture expanded cells may enable clone selection as a means of rational selection that will reduce the problem of batch-to-batch variation in culture expanded hMSC-like cell populations.

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IMAGES AND TABLES:

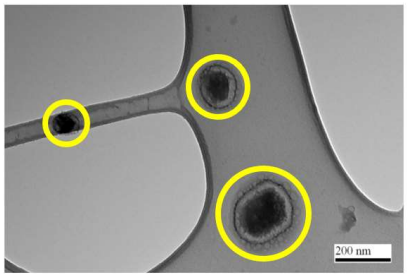

Figure 1. Transmission Electron Microscopy (TEM) image of mesenchymal stromal cell extracellular vesicles (MSC EVs) (50-200 nm) isolated using ultracentrifugation.

	Analytes with Positive Expression (Units: pg/mL)	Single Donor: Clones 1-4											
		Whole Non-Lysed EVs				Lysed EVs				Non-EV Secretomes			
		1	2	3	4	1	2	3	4	1	2	3	4
Higher Expression in Lysed EVs	Neuropilin-1	0	0	0	0	2112	1704	2255	227	578	375	311	217
	IFN-alpha	1	0	0	0	20	16	24	0	0	0	1	0
	CCL11/Eotaxin	0	0	0	0	120	0	46	0	0	0	0	0
Higher Expression in Non-Lysed EVs	IL-1ra/IL-1F3	230	1	56	1	1	181	1	1	0	0	0	0
	IL-1alpha/IL-1F1	1	0	63	0	0	1	0	0	0	0	0	0
Higher Expression in Non-EV Secretomes	IL-8/CXCL8	0	0	0	0	0	0	0	0	22	9	4	3
	MMP-2	0	0	0	0	12523	4440	761	498	46628	39489	33485	23064
	CCL2/JE/MCP-1	0	0	14	0	0	0	0	14	3748	943	941	319
	IL-6	0	0	0	0	0	0	1	0	538	266	458	187
	MMP-1	0	0	0	0	0	0	0	0	13	26	4	0
Variable Expression Amongst Fractions	FGF basic/FGF2/bFGF	0	0	2	0	41	69	56	82	42	39	55	70
	IL-13	0	0	36	256	0	131	0	0	0	131	131	0
	M-CSF	25	178	56	56	270	148	148	163	377	209	209	148
	HGF	13	0	0	0	178	42	108	73	433	127	266	136
	Lipocalin-2/NGAL	414	310	228	228	228	193	376	193	395	414	432	355
	CCL3/MIP-1 alpha	617	617	617	652	541	617	581	581	825	799	744	635

Range of Positive Expression per Analyte: Low — High

Table 1. Secretome profiles of 4 clonal MSC colonies derived from a single human donor. A panel of 48 analytes were assayed. These 16 analytes had detectable levels in either the EV or non-EV secretome. IFN-α, and Eotaxin were only found in the EV fraction. Neuropilin-1 was elevated in lysed EVs, with some Neuropilin-1 in the non-EV secretome. MMP-2 had the highest concentration in both the EV and non-EV fraction. IL-1ra and IL-1α were variably represented between clones in EV containing fractions. IL-6, IL-8 MCP-1, MMP-1 were only found in the non-EV secretome. FGF basic, IL-13, HGF, Lipocalin-2, and MIP-1α were variably represented in both EV and non-EV fractions.