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Human Macrophage Genetic Engineering

Jonathan D. Smith

Department of Cellular & Molecular Medicine, Cleveland Clinic, Cleveland OH

Jonathan D. Smith: smithj4@ccf.org

Macrophages, in addition to their role in innate immunity, play a central role in atherosclerosis as the precursors to foam cells in arterial lesions[1]. Macrophages express a variety of scavenger receptors that take up modified apoB-containing lipoproteins in a manner that is not subject to the negative feedback regulation observed for the LDL receptor, thus leading to the accumulation of excess cholesterol that is esterified and stored in lipid droplets. Macrophages also express *ABCA1* and *ABCG1*, and thus can efflux free cholesterol to apoA-I and HDL, respectively, in the first step of the reverse cholesterol transport pathway. Macrophages are widely studied in regard to atherosclerosis, modified lipoprotein uptake, and cholesterol efflux/reverse cholesterol transport, as well as to gain insight into pathways and mechanisms that might be leveraged for potential therapeutic strategies. Up until now, most of what we know about macrophage biology is from the study of murine macrophages. It is relatively simple to retrieve elicited peritoneal macrophages or culture bone-marrow derived macrophages from wild type, transgenic over expressing, or gene knockout mice. Thus, in vitro macrophage studies can be performed on cultured cells taking advantage of numerous available gene targeted mouse models. In addition, mouse bone marrow transplantation studies are often performed in order to distinguish the effects of gene targeting in hematopoietic-derived donor cells vs. host somatic tissue. There is one commonly used human monocytic cell line, THP-1, which can be differentiated into macrophage-like cells in culture, but the undifferentiated monocytic cells grow in suspension and are not easily selected clonally after genetic engineering. One can prepare primary human peripheral blood monocytes by density gradient centrifugation, which upon culturing for several days acquire a macrophage-like phenotype; however, it would be tedious to perform genetic engineering on these non-dividing primary cells.

In this issue of ATVB, Gupta et al. report a streamlined protocol to study genetically engineered human macrophages[2]. This protocol combines two existing methods along with the use of pluripotent human embryonic stem cells (hESC). The test gene in this case was *ABCA1*, which is essential in the formation of HDL and mutated in Tangier disease patients[3]. The *ABCA1* gene was disrupted in its coding region using CRISPR/Cas9 mediated targeted double strand breaks, which are repaired via non-homologous end joining to induce small insertion/deletion (indel) frameshift mutations. The HUES9 cell line was transiently transfected with plasmids encoding a CRISPR-GFP fusion protein along with one transcribing an *ABCA1* targeting guide RNA. The efficiency of this protocol relied on flow

Disclosures

None.

cytometry to select only the GFP-expressing cells. Of the 192 clonally derived cell lines screened, 76% had one or both *ABCA1* genes edited. These authors followed up with two cell lines each of unedited cells and those with bi-allelic frame shift mutations. The second stage of this protocol was the differentiation of these hESCs into CD14+ monocytes and then macrophages, adapting published methods[4, 5]. Western blots and cholesterol efflux to apoA-I were performed to assess ABCA1 protein expression and activity. Although both were markedly reduced in the bi-allelic edited cell lines, residual ABCA1 expression and activity were present, indicating that the cell lines were possibly contaminated with non-edited cells.

Another way to study genetic effects in human macrophages would be via making patient specific induced pluripotent stem cells (iPSC). Although, this first requires identifying and characterizing potentially rare mutations, there are several examples of these studies[5–7]. The methods described by Gupta et al. could also be applied to mouse embryonic stem cells, thus allowing researchers to make gene knockouts in mouse macrophages for which no mouse model exists. By adding a donor ssDNA to CAS9 and guide RNA transfections, one could select for homologous repair events, allowing one to make allele replacements to study not only protein coding changes, but also regulatory variants that might alter mRNA transcription, translation, or stability in human or murine macrophages. There is a great need for these types of studies, as genome wide association studies as well as functional genomic screens linking gene expression to genetic variation find many potential non-coding, putative regulatory variants that are in strong linkage disequilibrium with each other, and thus identification of the true causal regulatory variant requires functional testing[8–10]. Thus, it is likely that similar methodology as described by Gupta et al. will become widely adapted for functional genomic validation using gene edited stem cells induced to differentiate into a variety of tissue types.

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