### Title

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### Journal

Inflammation research : official journal of the European Histamine Research Society ... [et al.], 69(7)

### ISSN

1023-3830



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### Publication Date

2020-07-01

### DOI

10.1007/s00011-020-01349-7

Peer reviewed

HHS Public Access

Author manuscript

*Inflamm Res*. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

*Inflamm Res*. 2020 July ; 69(7): 631–633. doi:10.1007/s00011-020-01349-7.

# CHRFAM7A Reduces Monocyte/Macrophage Migration and Colony Formation In Vitro

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## Abstract

**Objective and Design:** CHRFAM7A is a uniquely human gene that encodes a dominant negative inhibitor of the α7 nicotinic acetylcholine receptor. We have recently shown that CHRFAM7A is expressed in human leukocytes, increases cell-cell adhesion, and regulates the expression of genes associated with leukocyte migration.

**Material:** Human THP-1, RAW264.7 and HEK293 cells.

**Methods:** Cell migration, cell proliferation and colony formation in soft agar to compare the biological activity of vector vs. CHRFAM7A transduced cells.

**Results:** We show that gene delivery of CHRFAM7A into the THP-1 human monocytic cell line reduces cell migration, reduces chemotaxis to monocyte chemoattractant protein, and reduces colony formation in soft agar.

**Conclusion:** Taken together, the findings demonstrate that CHRFAM7A regulates the biological activity of monocytes/macrophages to migrate and undergo anchorage independent growth in vitro.

### Keywords

monocyte migration; myeloid cell self-renewal; human-specific genes; α7- nicotinic acetylcholine receptor; dup α7- nicotinic acetylcholine receptor

## Summary:

We tested whether a uniquely human gene, CHRFAM7A, could be contributing to a human inflammatory response that is distinct from other species. Here, we demonstrate that CHRFAM7A expression reduces cell migration, and anchorage independent growth in soft



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agar. There is a biological consequence to the expression of CHRFAM7A that suggests species-specific pathways that differentially regulate monocyte/macrophage activity.

## Introduction:

The CHRFAM7A gene is a uniquely human variant of the CHRNA7 gene that encodes the α7 nicotinic acetylcholine receptor (α7nAchR), and may regulate cholinergic anti-inflammatory signaling pathways differently between species [1, 2]. Our previous studies characterizing the effects of CHRFAM7A expression demonstrated that the stable over-expression of CHRFAM7A in the human pre-monocyte THP-1 cell line alters gene expression pathways associated with leukocyte migration, focal adhesion and TGF-B signaling [3]. We also recently demonstrated that transgenic mice expressing human CHRFAM7A exhibit increased emergency myelopoiesis after severe injury that is associated with increased immune cell trafficking to the lung[4]. Here, we hypothesized that CHRFAM7A expression in mouse cells of monocyte/macrophage lineage might alter their activity and confer insight into uniquely human elements of inflammatory responsiveness.

## Materials and Methods:

See supplementary methods for complete detail.

## Results:

THP-1 cells that over-express CHRFAM7A demonstrated a time- and MCP-1 dose-dependent reduction in migration (Fig. 1A, B. There was no effect of CHRFAM7A expression on adhesion to the extracellular matrix proteins fibronectin, collagen, laminin, or fibrinogen (Fig. 1C) and no effect of CHRFAM7A expression on cell proliferation (Fig. 1D). Prior KEGG analyses of CHRFAM7-dependent gene expression in THP-1 cells demonstrated that forced expression of CHRFAM7A altered transforming growth factor-beta (TGF-B) expression [3]. Accordingly, we evaluated whether CHRFAM7A altered the TGF-B endpoint of cellular self-renewal using anchorage-independent colony formation [5]. As shown in Fig. 1E–G, CHRFAM7A expression decreased the ability of THP-1 cells to form colonies in soft agar compared to vector THP-1 cells, demonstrating that CHRFAM7A expression inhibits anchorage independent growth.

To determine whether the expression of CHRFAM7A affects anchorage independent growth of mouse macrophages expressing only the conserved CHRNA7, RAW264.7 cells expressed CHRFAM7A vs. ZsGreen controls were evaluated. CHRFAM7A expressed decreased anchorage independent growth in soft agar compared to control (Fig. 1H–J). We next tested human HEK293 cells that lack RIC3, a chaperone required for cholinergic receptor trafficking to the plasma membrane. Comparison of HEK293 cells expressing CHRFAM7A vs. controls that there no difference in colony formation (Fig. 1K–M).

## Discussion

Here, we demonstrate that CHRFAM7A over-expression in THP-1 cells reduced their migration and anchorage independent colony formation. Although the mechanism of this

effect is not known, we previously reported that CHRFAM7A expression increases expression of versican [6], a matrix proteoglycan know to mediate cell migration, as well as GPNMB [7], a transmembrane glycoprotein that promotes cell migration [3].

We also show that CHRFAM7A inhibits monocyte/macrophage colony formation. This in vitro supports our in vivo data in CHRFAM7A transgenic mice demonstrating that CHRFAM7A increases emergency myelopoiesis and trafficking of myeloid cells to sites of tissue inflammation [4]. The fact that colony growth is inhibited in CHRFAM7A transduced RAW264.7 cells establishes that the biological activity of CHRFAM7A remains intact when inserted into mouse cells. There was also a CHRFAM7A-dependent decrease in RAW264.7 cell colony formation that was not observed in CHRFAM7A-transduced HEK293 cells which lack the RIC3 chaperone[8] required for cholinergic receptor activity. This further suggests that CHRFAM7A may be interacting with α7nAchR receptor function on the cell surface.

The finding that there is a biological consequence to the expression of a uniquely human gene like CHRFAM7A and that its effect can be conferred to mouse cells, suggest that species-specific pathways recently evolved to differentially regulate monocyte/macrophage activity, and as such, the response to inflammation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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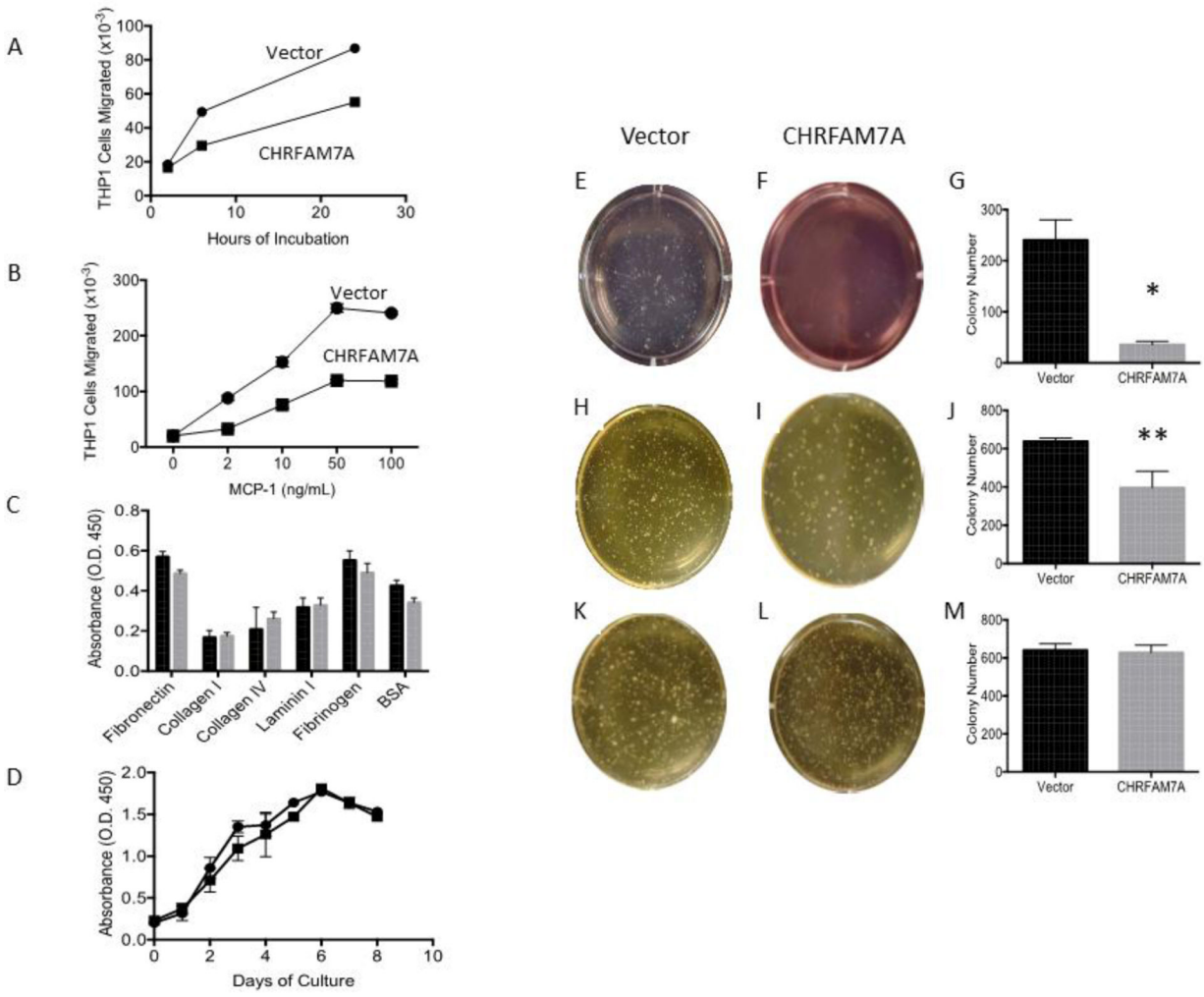


**Figure 1- CHRFAM7A expression alters the biologic activity of monocyte/macrophages.**

CHRFAM7A expression (square) decreased THP-1 cell migration to MCP-1 compared to control (circle) in a (A) time and (B) dose-dependent manner (6 hour timepoint). CHRFAM7A had no effect on THP-1 cell (C) adhesion to various extracellular matrix proteins (black bar = vector, grey bar = CHRFAM7A) or (D) proliferation (circle = vector, square = CHRFAM7A). THP-1 colony formation in soft agar was measured in (E) vector and (F) CHRFAM7A over-expressing cells. (G) Quantification of colonies observed after 3-week incubation demonstrated that CHRFAM7A inhibits THP-1 cell self-renewal. (H-J) CHRFAM7A decreased RAW264.7 colony growth compared to vector. (K-M) There was no difference in colony formation when CHRFAM7A was introduced into HEK293 cells that lack the RIC3 chaperone protein required for cholinergic receptor activity. * $p<0.05$, ** $p<0.01$.