ANALYSIS OF RHOU AND RHOV EXPRESSION IN MULTIPLE MYELOMA REVEALS A POSSIBLE CORRELATION WITH BONE MARROW DEPENDENCE

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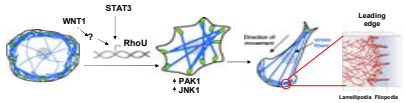
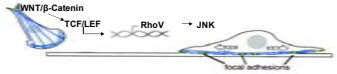
BACKGROUND

Rho GTPases, in their active GTP-bound state, interact with effector proteins controlling many biological processes: cytoskeletal regulation, membrane trafficking, cell adhesion, cell polarization, transcriptional activity, apoptosis, and cell proliferation.

RhoU is known to be transcriptionally induced by STAT3 in the JAK/STAT pathway, and is believed to be also transcriptionally induced by the non-canonical Wnt pathway affecting cell polarity, directional sensing, cell adhesion and migration.



RhoV is transcriptionally induced by the Wnt canonical pathway (via GSK3β/β−Catenin) leading to cell-cell or cell-extra cellular matrix adhesion, and is somehow implicated in cytoskeleton reorganization by activation of the JNK pathway.



Due to their spontaneous activation, they are normally expressed at very low levels in various tissues and organs.

In Multiple Myeloma (MM), adhesion between primary malignant plasma cells and stromal cells protects MM cells from chemotherapy. Knowing that RhoU and RhoV can alter cell adhesion, actin dynamics and cell motility, we hypothesize that changes in their expression, and thus activity, may lead to a remodeling of MM associated bone marrow niches.

OBJECTIVES

In this study we aimed to check RhoU and RhoV expression and actin cytoskeleton conformation in normal versus MM malignant plasma cells. We also evaluated if stroma derived soluble factors, known to be important MM survival factors, had an impact in the expression of these GTPases.

MATERIALS & METHODS

qRT-PCR was performed using cDNA from different MM cell lines or malignant plasma cells (PCs) from MM and Plasma Cell Leukemia (PCL) patients. cDNA from normal B cells of healthy donors was used as control. GAPDH was used as a reporter gene.

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of healthy donors by Ficoll separation. Normal B cells were purified from PBMCs using "EasySep human B cell isolation kit". MM plasma cells were isolated from bone marrow or peripheral blood of patients using "EasySep human whole blood and bone marrow CD138 positive selection kit".

Fluorescence microscopy was performed using Zeiss LSM 700 microscope. Immunostaining was done with Phalloidin Alexa fluor 594 (Abcam), rabbit anti-RhoU or RhoV (Abcam), and goat anti-rabbit Alexa fluor 488 (Life Technologies). Slides were mounted with mounting media complemented with DAPI.

RESULTS

Overexpression of RhoU and RhoV seems to correlate with bone marrow dependence

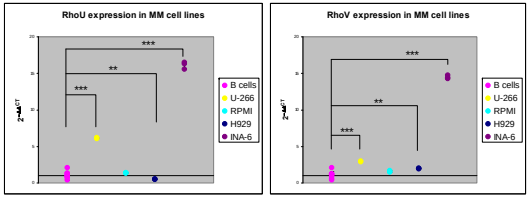


Figure 1: Relative expression of RhoU and RhoV in MM cell lines as compared to normal B cells from healthy donors: U-266 and RPMI8226 are MM cell lines both been obtained from peripheral blood of patients with plasma cell leukemia (U-266 has an autocrine production of IL-6); H929 derived from pleural effusion; and INA-6 from bone marrow (stroma/IL-6 dependent). * p<0.05 ** p<0.01 *** p<0.001

RhoU and RhoV are overexpressed in bone marrow derived malignant PCs but not in PCL cells

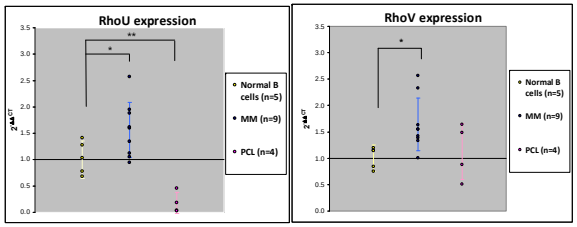


Figure 2: Relative expression of RhoU and RhoV in freshly extracted PCs from MM patients as compared to normal B cells from healthy donors. Samples named MM are PCs from bone marrow extracts of MM patients, while samples named PCL derive from the peripheral blood of patients in the stage of Plasma Cell Leukemia. * p<0.05 ** p<0.01 *** p<0.001

IL-6, a MM survival factor, induces early up-regulation of RhoU

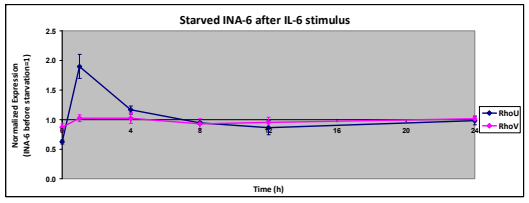


Figure 3: INA-6 cells were starved from IL-6 for 12 hours before stimulation. The graphic shows the relative expression of RhoU and RhoV after stimulus with IL-6, normalized over RhoU/RhoV levels before starvation. n=3; p<0.01

Conditioned media from stroma cell culture induces up-regulation of RhoU to the same extent as IL-6 stimulus

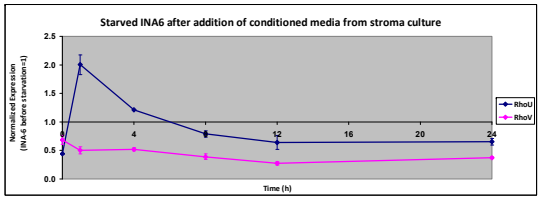


Figure 4: INA-6 cells were starved from IL-6 for 12 hours before stimulation, stroma cells where cultured in RPMI+10%FCS for 12hours. The image shows the relative expression of RhoU and RhoV in INA-6 after addition of conditioned media from stroma cell culture, normalized over RhoU/RhoV levels before starvation. n=3; p<0.01

RhoU localizes to nuclear organelles of MM but not PCL cells

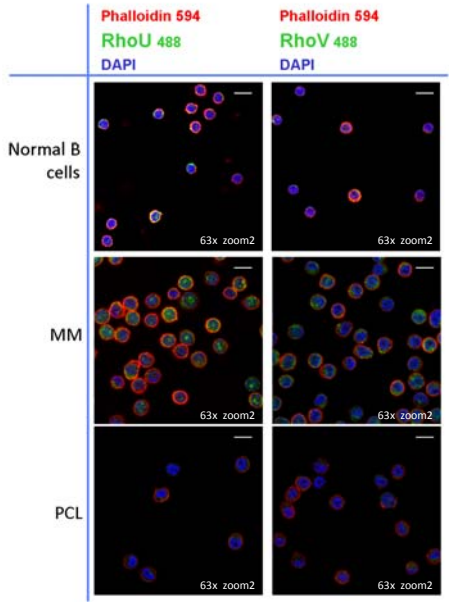


Figure 5: Immunofluorescence images of freshly purified PCs from patients and B cells from healthy donors stained for RhoU/RhoV (green), actin cytoskeleton (red) and nucleus (blue). Samples named MM are PCs purified from bone marrow extracts of MM patients, while samples named PCL derive from the peripheral blood of patients in the stage of Plasma Cell Leukemia. These images are representative of the acquisition of 5 samples of normal B cells, 5 samples of MM plasma cells and 3 samples of PCL. Bars displayed are 10um long.

SUMMARY

When compared to normal B cells, most MM cell lines display higher levels of both RhoU and RhoV that are more evident on IL-6/ stroma dependent cells. Stroma-independent cell lines have a lower expression of both proteins but RhoU is unexpectedly expressed at high levels in peripheral blood-derived cell line U-266 (>5 fold changes), possibly due to its autocrine IL-6 production.

Freshly purified plasma cells from MM patients also exhibit significantly higher levels of both proteins. Interestingly, plasma cells from peripheral blood of PCL patients displayed normal levels of RhoV and extremely low levels of RhoU which suggests that the expression of these GTPases could be MM stage dependent.

Soluble factors important for MM cell survival, as IL-6 alone or stroma derived factors, are able to actively regulate the expression of RhoU in stroma/IL-6 dependent cell line. Other stimulus tested as TNFα had no effect in the expression of either protein (results not shown).

While RhoU seems to be modulated by soluble factors expressed by stroma cells, RhoV is most likely dependent on cell-cell contact.

Immunofluorescence imaging has shown an interesting location of RhoU in nuclear organelles in MM patient cells; unraveling its location may give new insights about its function in malignant plasma cells.

CONCLUSIONS

From these results we hypothesize a correlation between RhoU/RhoV expression and bone marrow dependence.

Since these proteins can alter cell adhesion, actin dynamics and cell motility, changes in their expression might determine differences in MM-associated bone marrow niches. For these reasons we strongly believe that these could be potential determinants of MM sensitivity to chemotherapy .

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