Effect of the microenvironment on alternative splicing in colorectal cells

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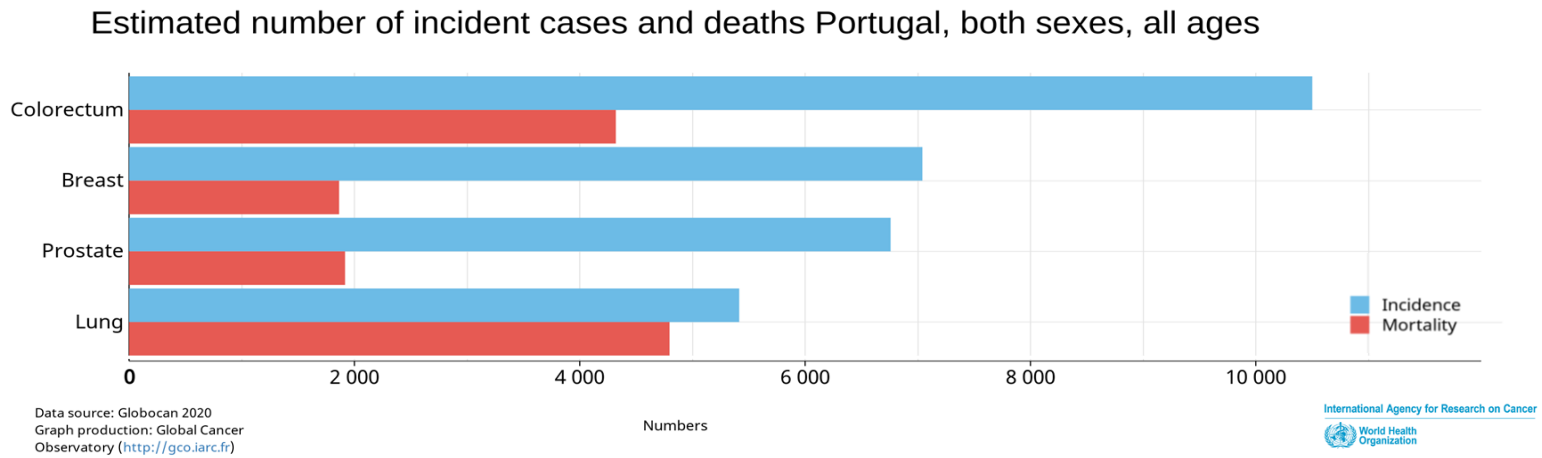
INTRODUCTION

- Colorectal cancer is one of the most prevalent tumors worldwide and the second cause of cancer mortality in Portugal

- Heterogeneous disease

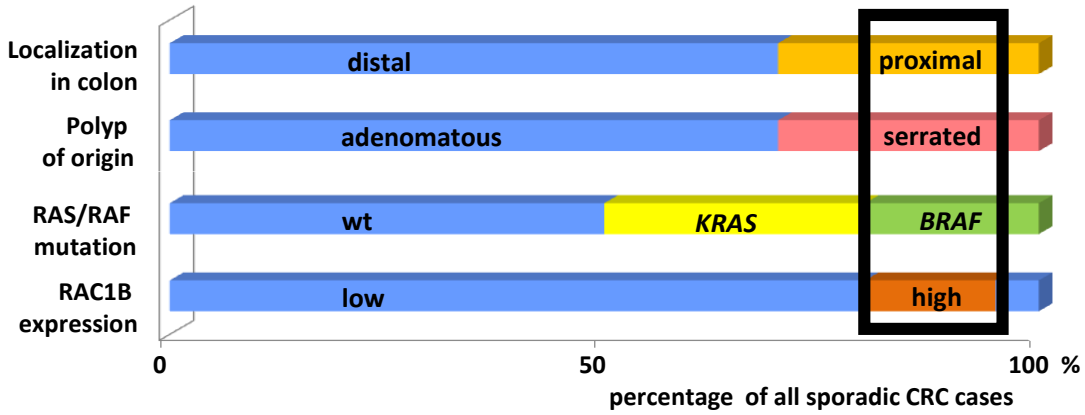


- RAC1B is an alternative splicing variant of the signaling-competent GTPase RAC1;

- Higher activation level than RAC1 with preferential stimulation of cell survival;

- Overexpressed in a subtype of colorectal tumors (BRAF-V600E-positive);

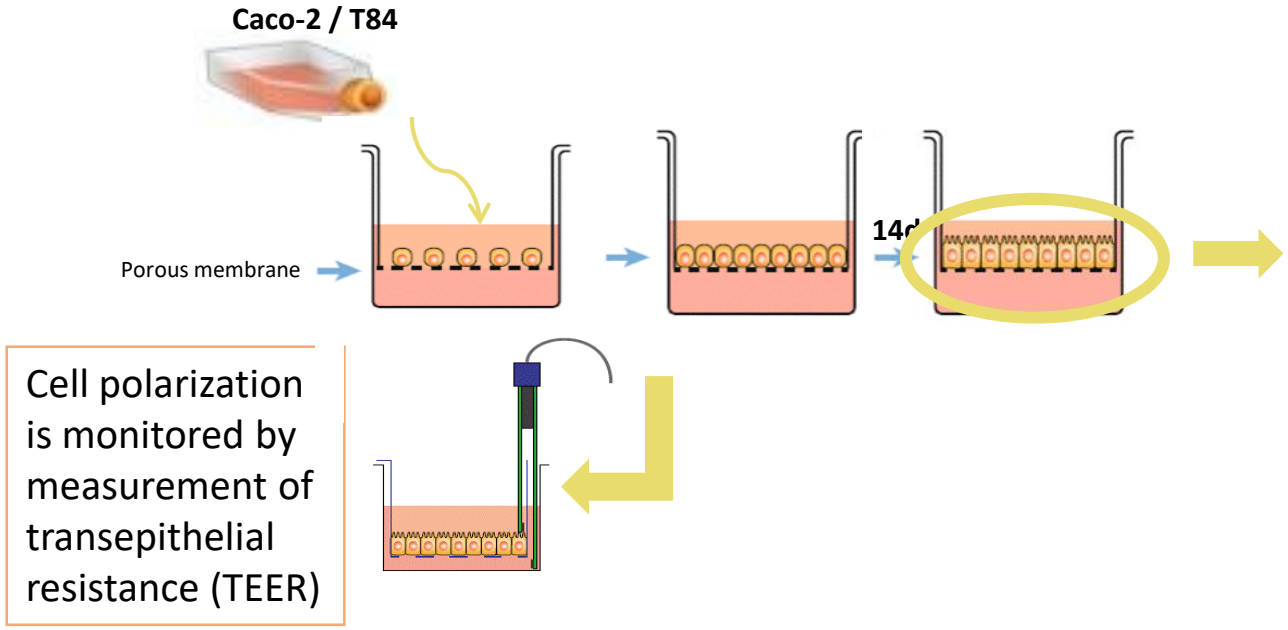
- Colon inflammation seems to be a trigger for increased RAC1B expression;

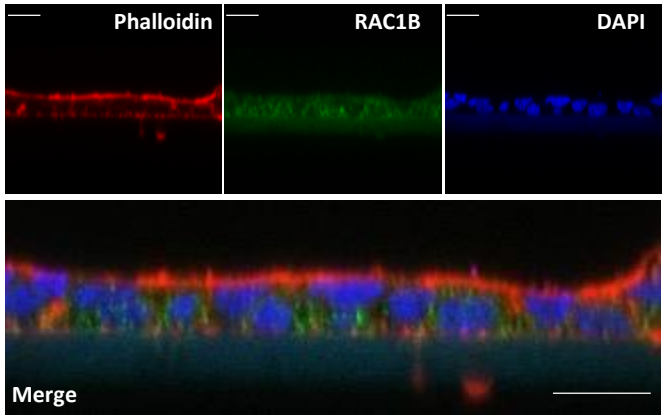


OBJECTIVES

Test whether tumor cells respond to a pro-inflammatory microenvironment by increasing biomarker RAC1B and identity the underlying molecular signal

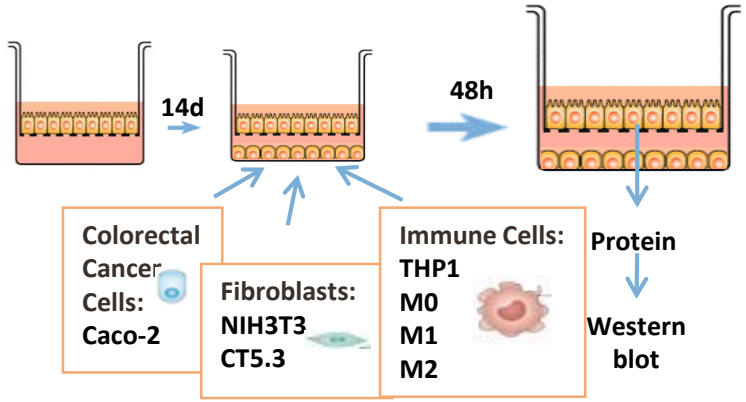
MODEL



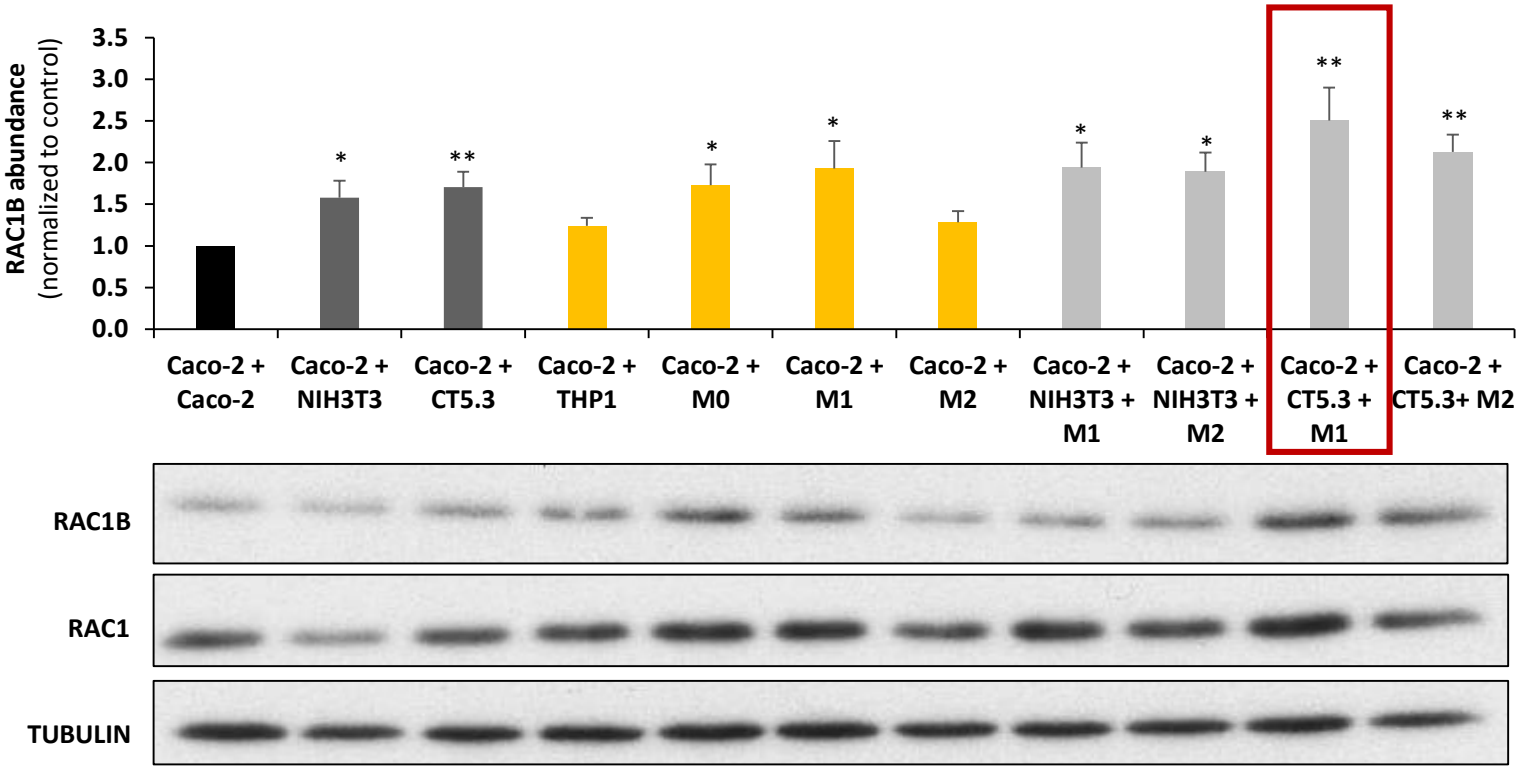


- Epithelial organization and polarization

RESULTS

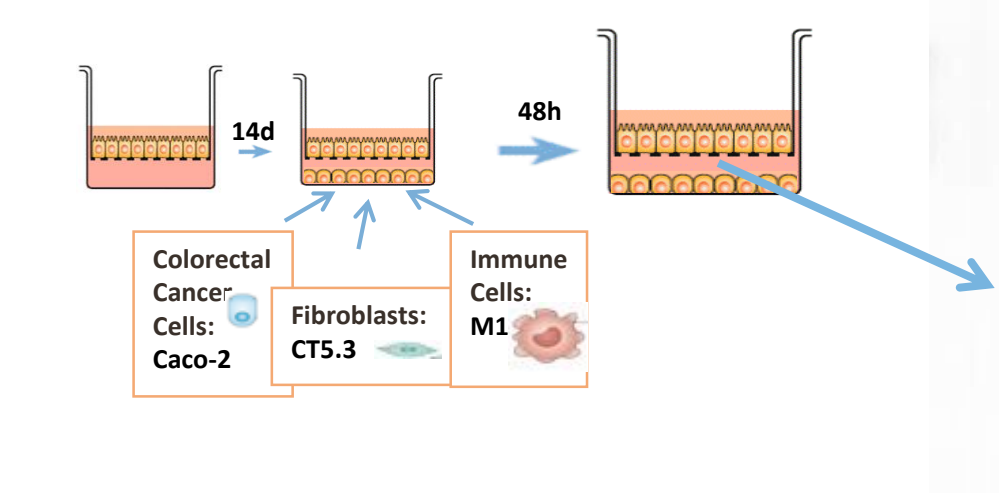


CT5.3 – cancer associated fibroblasts (CAFs)
M1 – pro-inflammatory macrophages
M2 – anti-inflammatory macrophages



- Co-culture of polarized Caco-2 with fibroblasts or macrophages or both led to an increase in RAC1B
- Increase in RAC1B was most significant with CT5.3 fibroblasts and M1 macrophages (pro-inflammatory condition)
- Comparable data obtained with polarized cell line T84, but not with non-polarized cells

48 h of co-culture - Identify which cytokines were released by stromal cells and responsible for the RAC1B protein increase in Caco-2 cells



Human inflammation antibody array

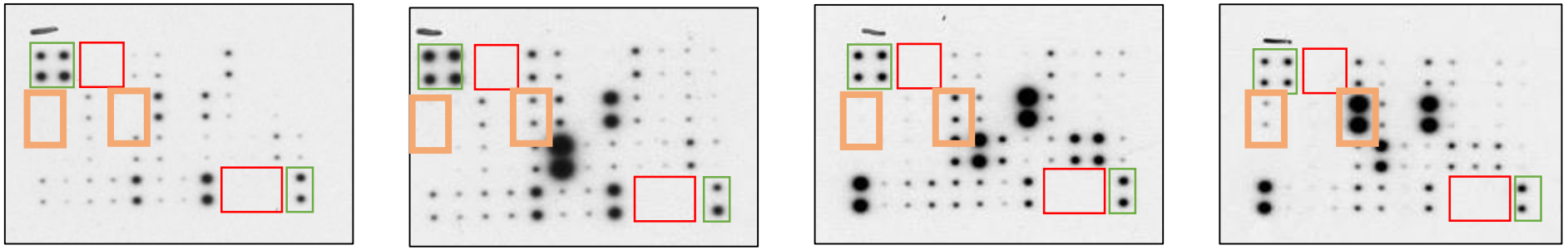
RESULTS

Caco-2 + Caco-2

Caco-2 + CT5.3

Caco-2 + M1

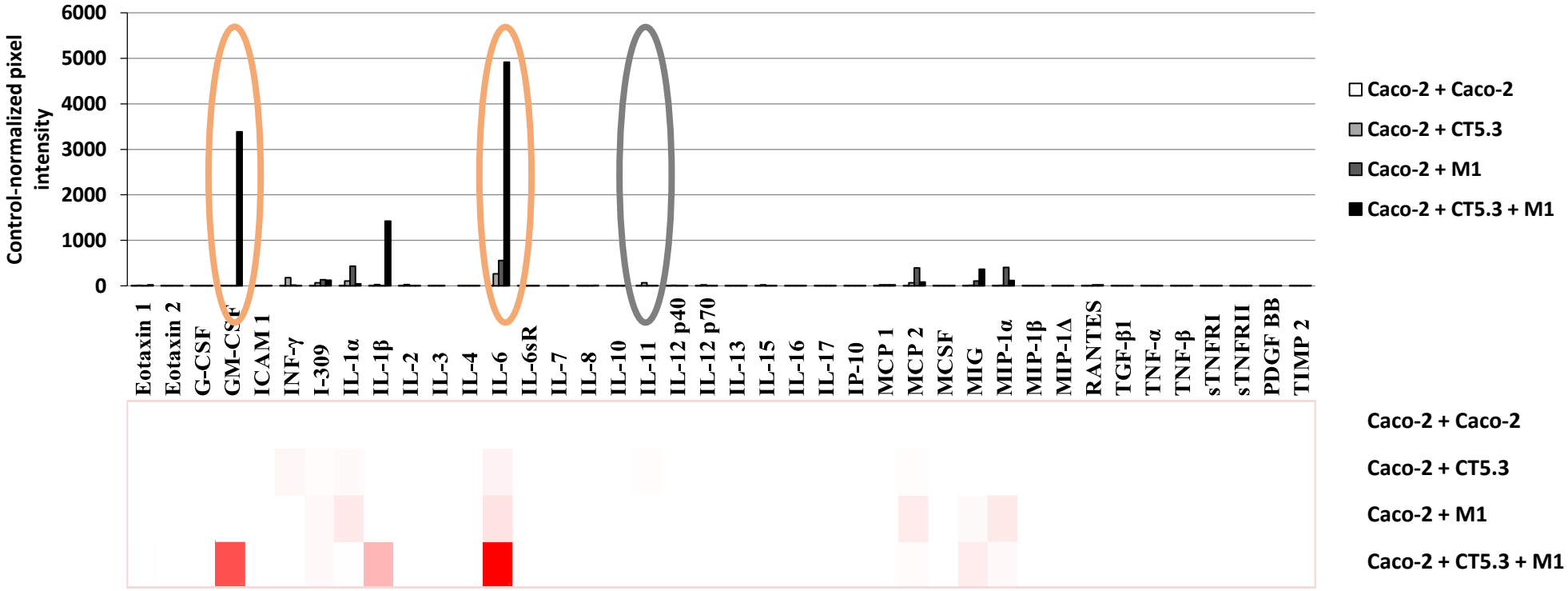
Caco-2 + CT5.3 + M1



Positive control spots

Negative control spots

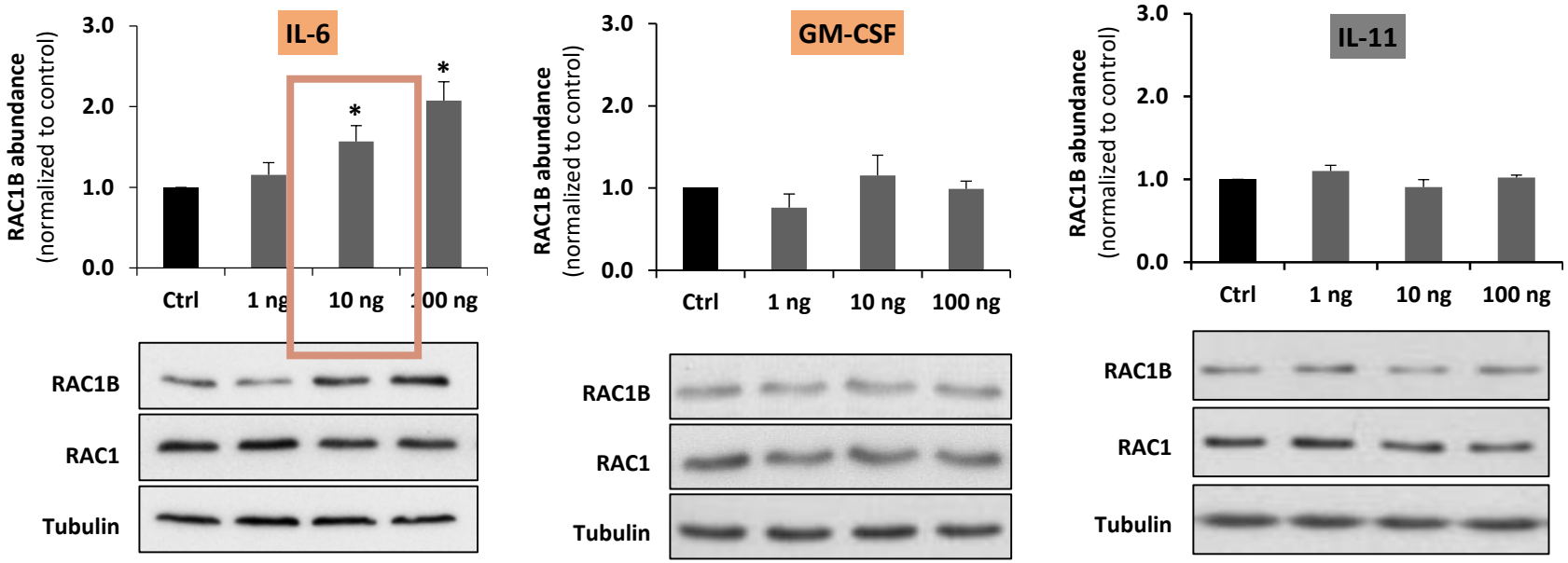
Human inflammation antibody arrays (AAH-INF-3-8; RayBiotech Inc.)



GM-CSF
IL-6

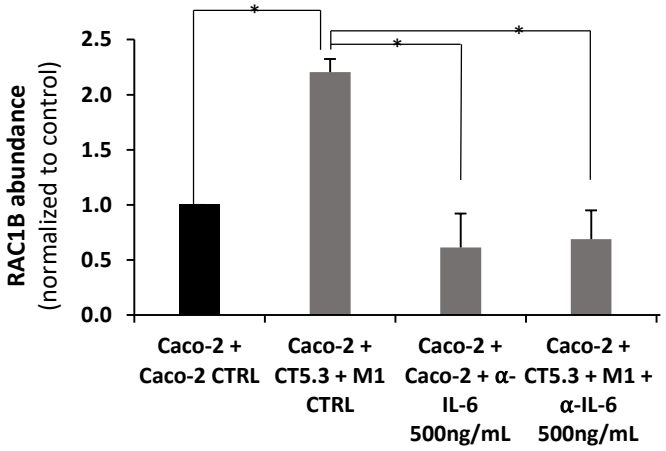
IL-11

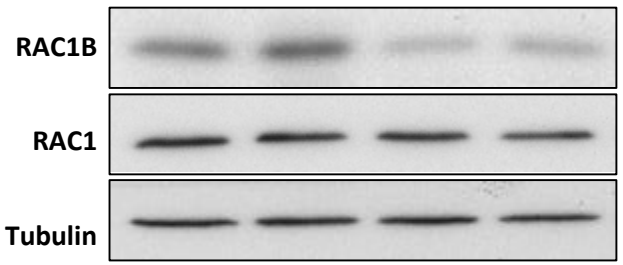
RESULTS

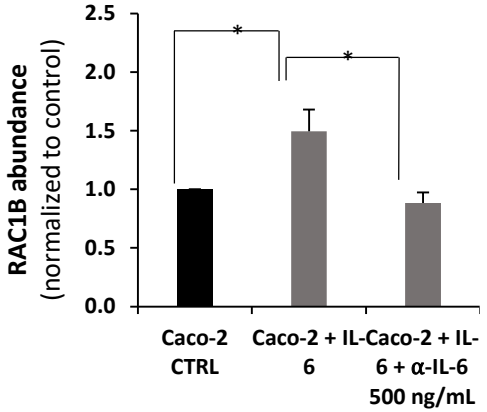


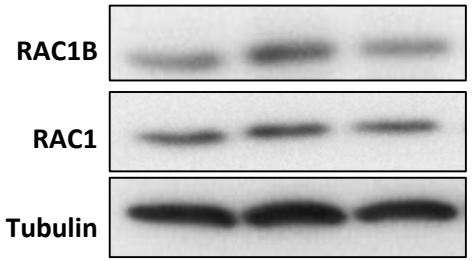
- IL-6 increased the expression of RAC1B protein in a dose-dependent manner
- GM-CSF, as well as the IL-11 control, caused no significant effect

RESULTS





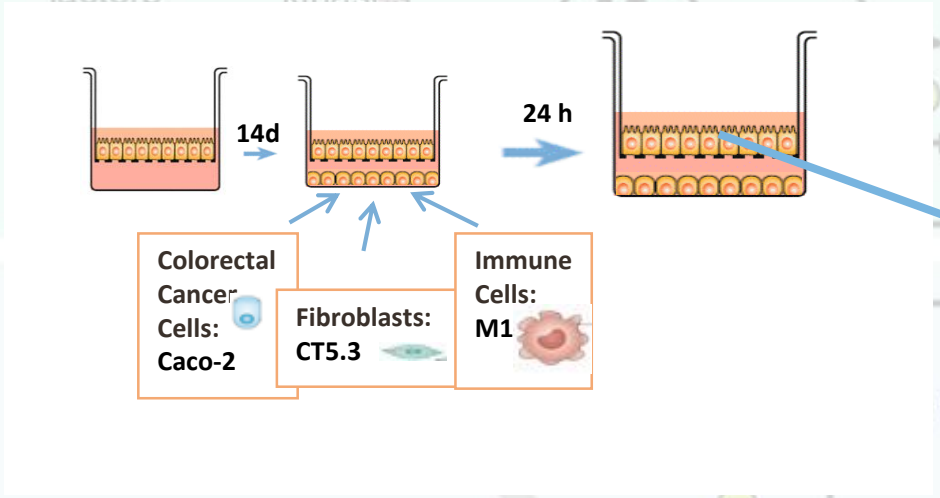




- The increase of RAC1B protein levels in Caco-2 cells was blocked by the addition of 500 or 1000 ng/mL of the anti-human IL-6 antibody

Causal relationship between the array-identified cytokine IL-6 and the increase in RAC1B

Inflammatory TME → **Tumor-promoting condition** → **Provides survival signals** → **Cancer cells respond with changes in gene expression: such as Alternative Splicing (AS)**

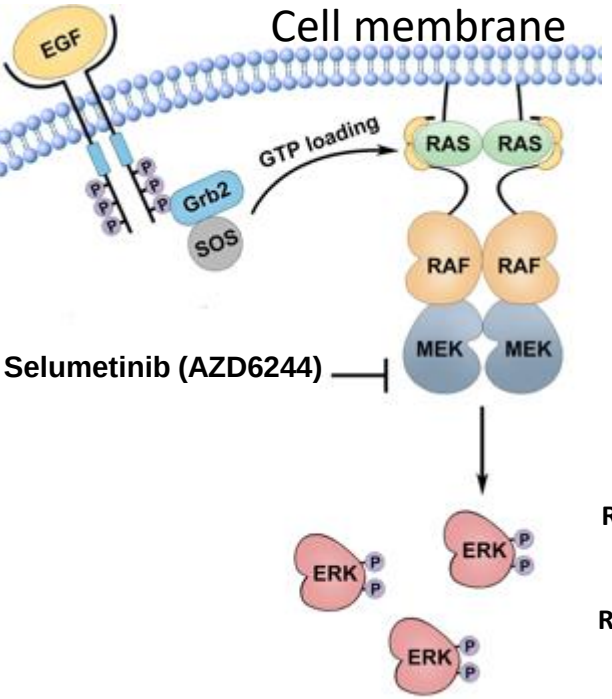


Human phosphoprotein antibody array

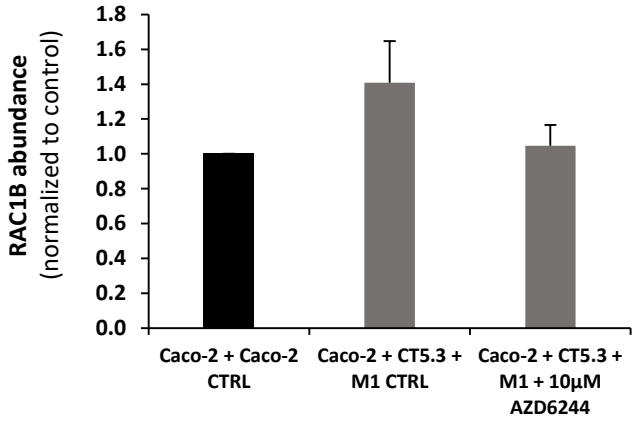
24 h of co-culture - Identify early protein phosphorylation events that regulate the response of Caco-2 cells

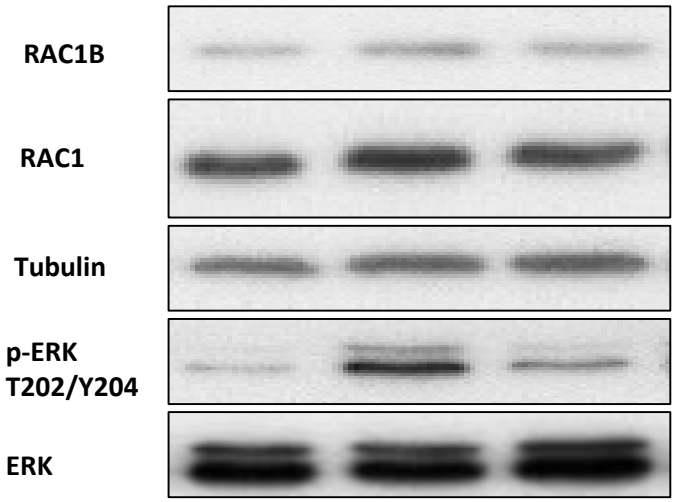
Main identified targets:
- ERK
- STAT3

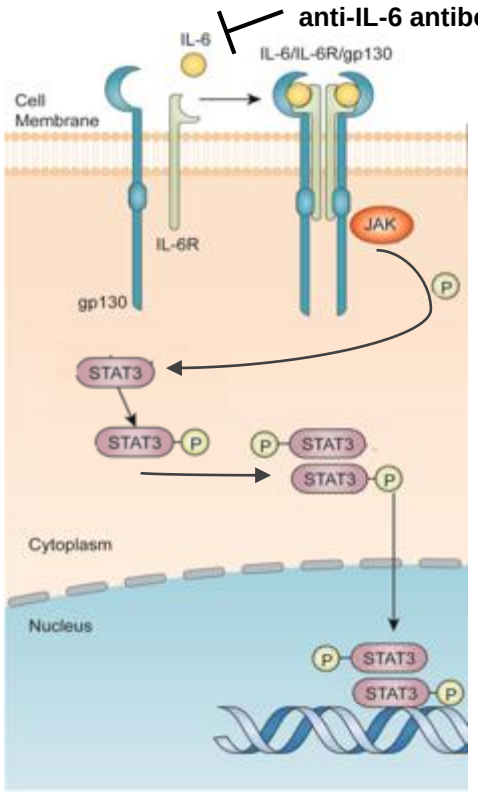
RESULTS



Adapted from Yuan, J., *et al. J Hematol Oncol* 13, 113 (2020)







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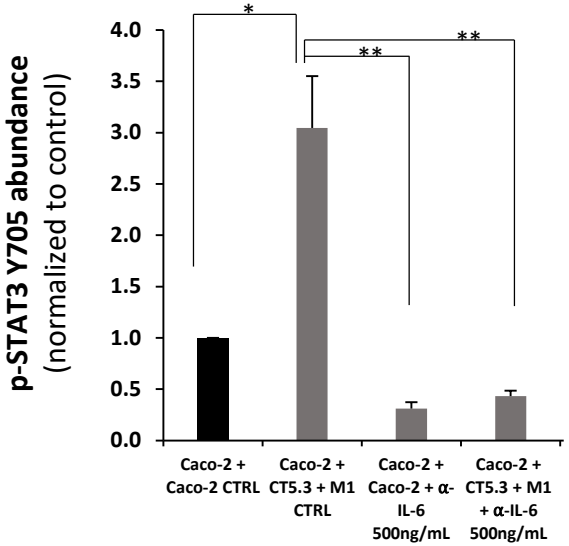
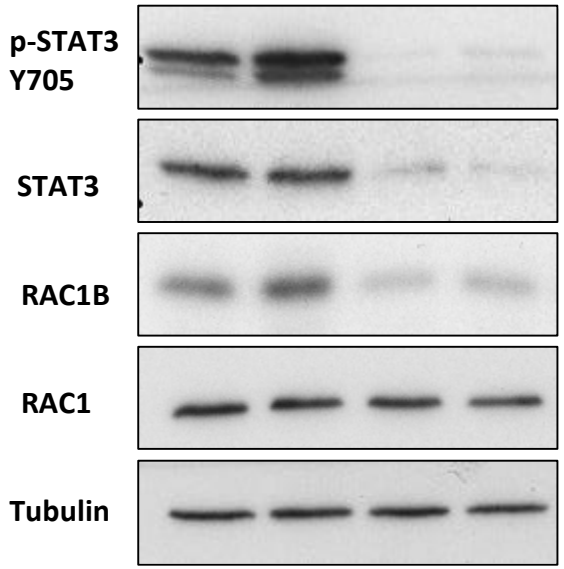
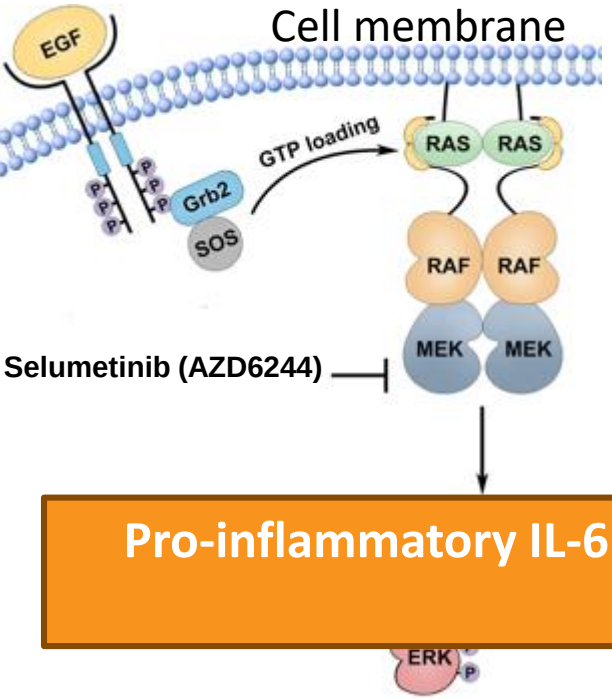




- Selumetinib not only decreased the phosphorylation of ERK1/2 but also prevented the co-culture induced increase in RAC1B
- Effect of IL-6 neutralization led to a decrease of STAT3 Y705 phosphorylation, STAT3 and RAC1B protein expression

RESULTS



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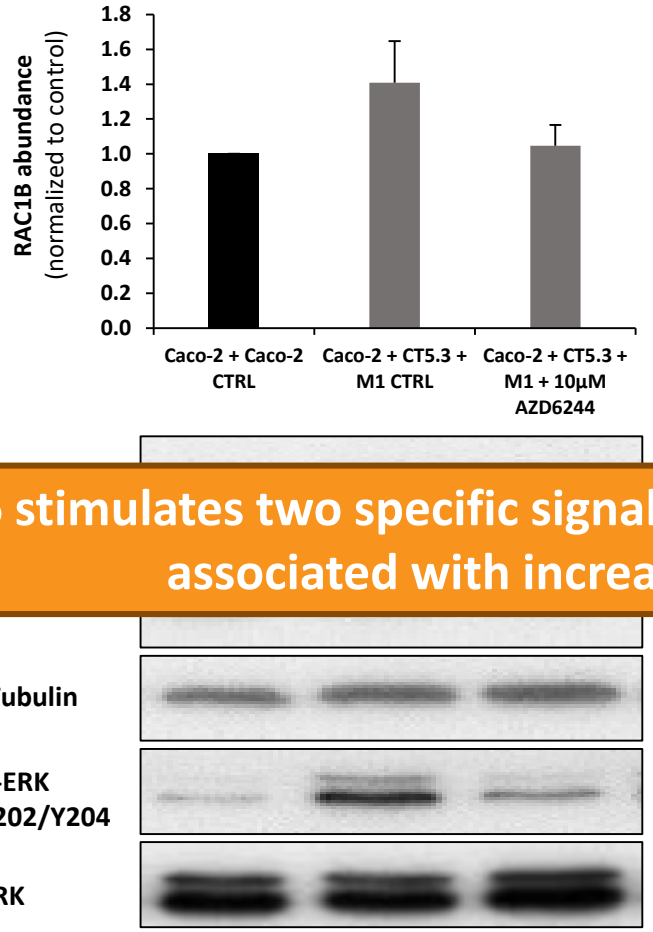

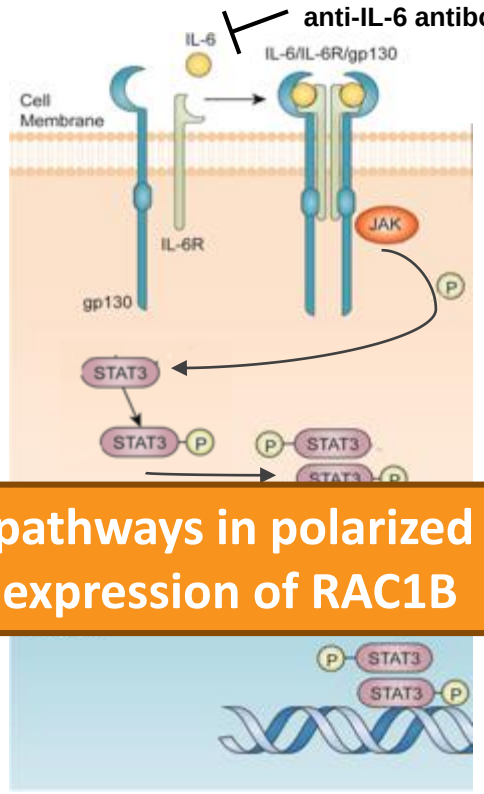

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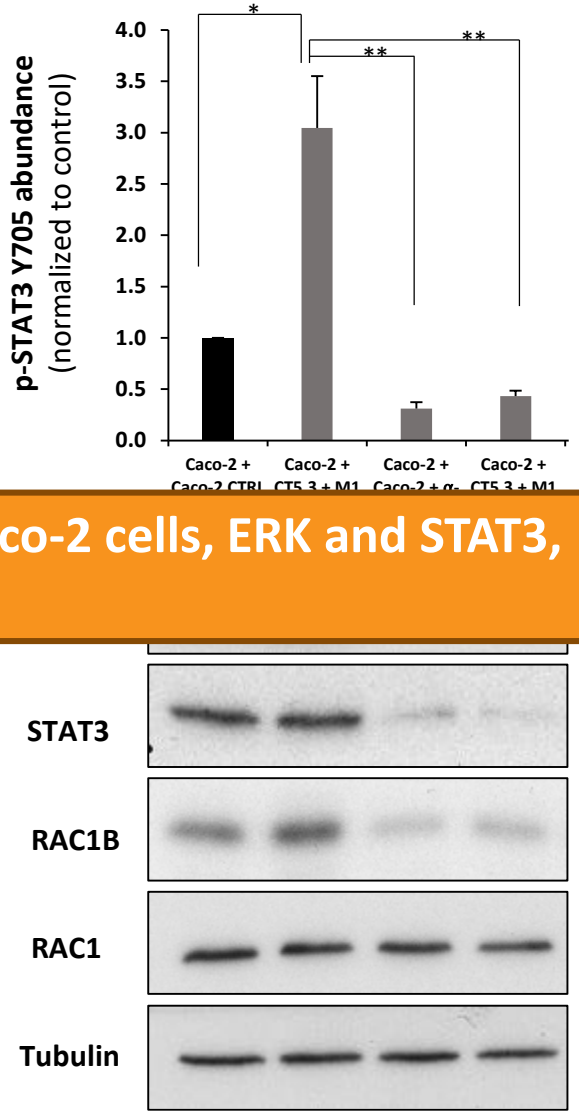
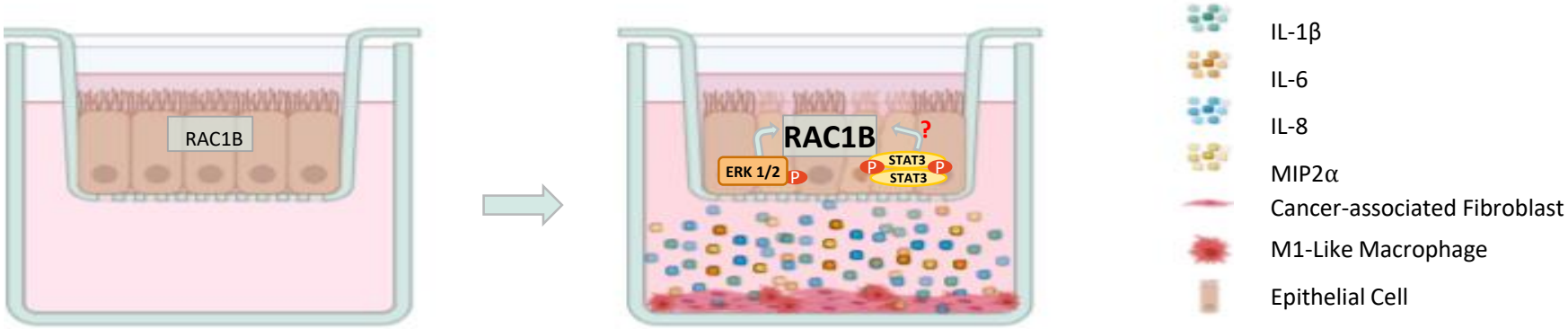


Pro-inflammatory IL-6 stimulates two specific signaling pathways in polarized Caco-2 cells, ERK and STAT3, associated with increased expression of RAC1B

- Selumetinib not only decreased the phosphorylation of ERK1/2 but also prevented the co-culture induced increase in RAC1B
- Effect of IL-6 neutralization led to a decrease of STAT3 Y705 phosphorylation, STAT3 and RAC1B protein expression

CONCLUSIONS

- A molecular connection between inflammatory conditions and the increase in RAC1B was identified
- IL-6 stimulates activation of STAT3 and MEK/ERK signaling pathways in Caco-2 cells



FUTURE PERSPETIVES

- Study the underlying signaling pathways in more detail to identify therapeutic targets
- Determine global gene expression response to IL-6 and validate in organoid or tumor samples

Discover new biomarkers for early tumorigenic stages or provide novel therapeutic targets of CRC

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