IDENTIFICATION OF A RECEPTOR INVOLVED IN THE CHRONIC DELETERIOUS EFFECTS OF TWO ENVIRONMENTAL POLLUTANTS IN A PRECANCEROUS BREAST CELL LINE

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INTRODUCTION

Growing evidence indicates that exposure to environmental carcinogens may increase the risk of sporadic breast cancers, so it is of most importance to decipher the role of environmental pollutants in the chronic carcinogenesis of human breast epithelia and their contribution in the development of this cancer. In this study we first characterized the **MCF10 breast tumour progression model**, that mimics progression from benign to premalignant or fully malignant phenotype [1]. Two environmental factors possessing distinct mechanisms of toxicity were chosen for chronic and low doses exposure of the **MCF10AT1** precancerous breast cell line of the tumour progression model. Cells were exposed to low-doses of an endocrine disruptor, **ED**, or a genotoxic compound, **GT**, or both of them for 60 days. We characterized the cellular and molecular phenotypes developed by the exposed cells, and investigated the candidate role of a specific receptor X (**RX**), in mediating their deleterious effects.

METHODS

Establishment of the chronically exposed cellular model: MCF10AT1 cells were chronically exposed to the indicated concentration of chemicals during 60 days in phenol red-free DMEM/Ham's F12 medium supplemented with 5% steroid-depleted, dextran-coated and charcoal-treated horse serum and 100 ng/mL cholera enterotoxin,10 mg/mL insulin, 0.5 mg/mL hydrocortisol, 20 ng/mL epidermal growth factor. Media and treatment were changed every 2 days. Unexposed MCF10CA1A.cl1 were used as control.

Characterization of the cellular and molecular phenotypes developed by the exposed cells: RTQ-PCR, Western Blot analysis, Anchorage independent growth and Mammospheres formation efficiency assays were performed.

1. The increasing malignant potential of the MCF10 model is associated with increased anchorage-independent growth and mammospheres formation efficiency

The MCF10 tumour progression model

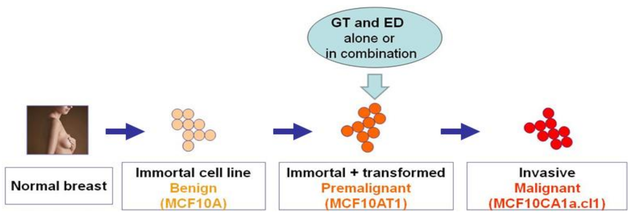
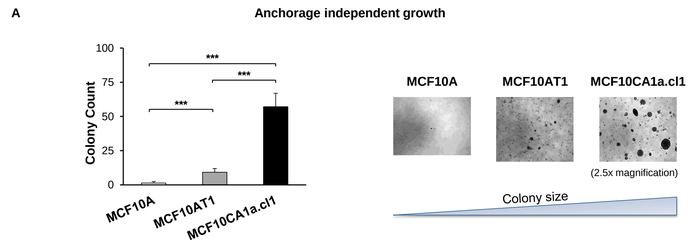


Fig.1: The MCF10 cell series. The MCF10A cells are spontaneously immortalized, non-malignant cells obtained from a patient with fibrocystic disease [2]; MCF10AT1 is a premalignant cell line produced by transfection of MCF10A with T24 *Ha-ras* which generates carcinomas in ~25% of xenografts [3-4]. MCF10CA1a.cl1 is the most malignant and aggressive cell line from the MCF10 series: it was derived using a strategy of passages of MCF10AT xenografts, and generates carcinomas with 100% efficiency [5].

Characterization of the MCF10 tumour progression model

The increasing malignant potential of the tumour progression model is associated with increased anchorage-independent growth, a hallmark of malignant cells that is known to be associated with aggressiveness and metastasis



The increasing malignant potential of the tumour progression model is associated with increased mammospheres formation, representative of self-renewal, a key feature of cancer-initiating cells

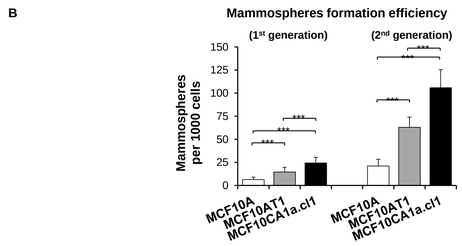
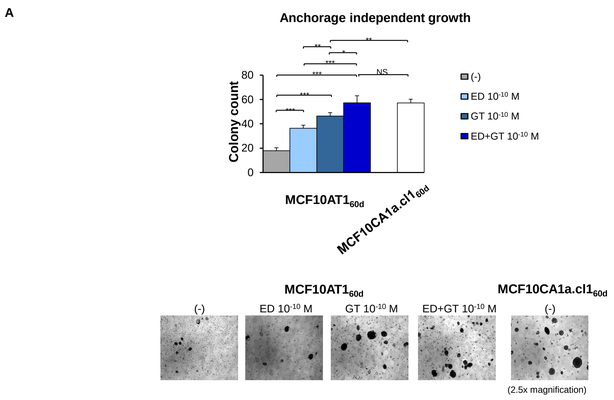


Fig.2: (A) Anchorage independent growth was assessed by Soft Agar assay. Single-cell suspensions of MCF10A, MCF10AT1 and MCF10CA1a.cl1 cells were seeded in soft agar, and colonies were counted after 3 weeks of incubation. **(B)** Single-cell suspensions of MCF10A, MCF10AT1 and MCF10CA1a.cl1 cells were seeded in non-adherent mammosphere culturing conditions. Two weeks later, mammospheres were counted, collected, trypsinated, and replated in non-adherent culturing conditions. Results are mean ± SD of 3 independent experiments. ***: p< 0.001 (Student t-test).

2. Long-term and low-doses exposure of environmental factors affects the aggressiveness of the precancerous cell line MCF10AT1

Chronic and low-doses exposure of ED and/or GT increases anchorage-independent growth



Chronic and low-doses exposure of ED and/or GT increases mammospheres formation efficiency

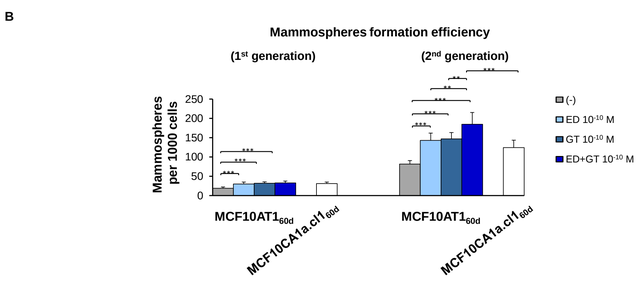
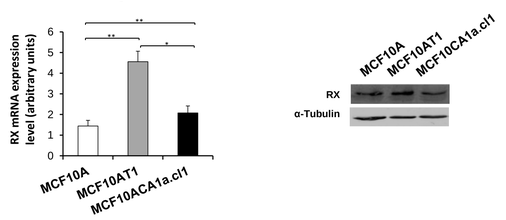


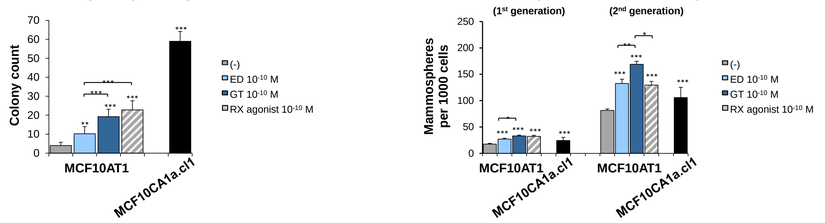
Fig 3: (A) After 60 days of chronic treatment with ED and/or GT (10^{-10} M), single-cell suspensions of MCF10AT1 cells were seeded in soft agar. Colonies were counted after 3 weeks of incubation. **(B)** After 60 days of chronic treatment with ED and/or GT (10^{-10} M), single-cell suspensions of MCF10AT1 cells were seeded in non-adherent mammosphere culturing conditions. Two weeks later, mammospheres were counted, collected, trypsinated, and replated in non-adherent culturing conditions (mean ± SD of 3 experiments). *: p<0.05, **: p<0.01, ***: p< 0.001 (Student t-test). Unexposed MCF10CA1a.cl1 cells were used as controls.

3. The deleterious effects of the environmental factors are directly or indirectly mediated by the receptor RX

The RX receptor is present in the MCF10 tumour progression model



An RX agonist molecule is able to induce anchorage independent growth and mammospheres formation



An RX antagonist molecule completely inhibits anchorage independent growth and mammospheres formation induced by the RX agonist, ED and GT

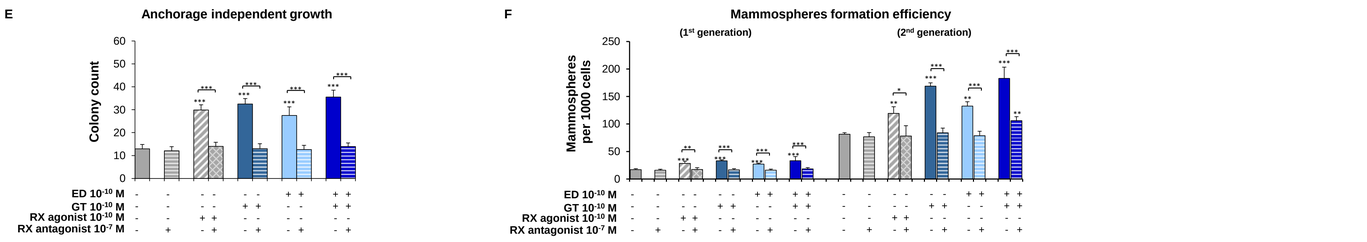


Fig 4: RTQ-PCR **(A)** and Western Blot analysis **(B)** of RX mRNA and protein expression in the MCF10 cells. **(C)** single-cell suspensions of MCF10AT1 cells were seeded in soft agar in the presence of ED, GT and RX agonist 10^{-10} M and colonies were counted after 3 weeks of incubation. **(D)** Single-cell suspensions of MCF10AT1 cells were seeded in non-adherent mammosphere culturing conditions in the presence of ED, GT and RX agonist 10^{-10} M. Two weeks later, mammospheres were counted, collected, trypsinated, and replated in non-adherent culturing conditions. Anchorage independent growth **(E)** and mammospheres formation efficiency **(F)** were then tested in the presence of the RX antagonist 10^{-7} M (mean ± SD of 3 independent experiments). *: p<0.05, **: p<0.01 ***: p< 0.001 (Student t-test).

4. The use of a specific antagonist for the receptor RX is sufficient to prevent the chronic deleterious effects of the environmental pollutants in the precancerous cells

The RX antagonist is able to prevent the chronic deleterious effects of the environmental factors on anchorage independent growth and mammospheres formation in the MCF10AT1 cells

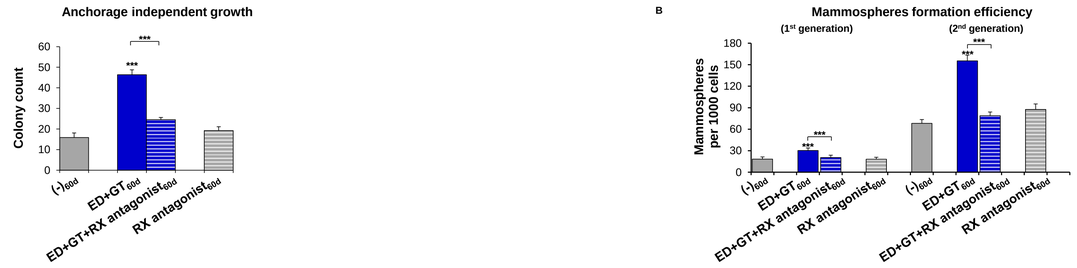


Fig 5: After 60 days of chronic treatment with the combination of ED and GT (10^{-10} M) in the presence or not of the specific RX antagonist (10^{-7} M), single-cell suspensions of MCF10AT1 cells were seeded in soft agar **(A)** or in non-adherent mammosphere culturing conditions **(B)** (mean ± SD of 3 experiments). ***: p< 0.001 (Student t-test).

CONCLUSION

This study highlights that:

- Chronic and low-doses exposure of environmental factors affects the aggressiveness of the precancerous cell line MCF10AT1
- The RX receptor is directly or indirectly involved in mediating the deleterious effects of the environmental pollutants in the precancerous cell line MCF10AT1
- Blocking the receptor RX with a specific antagonist is sufficient to prevent the deleterious effects of the chronic and low-dose exposure to the environmental factors

References:

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