MULTI-TARGETED ANTICANCER ACTIVITY OF HUMAN AMNIOTIC MEMBRANE HOMOGENATE ON VARIOUS CANCER CELLS

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Introduction

Based on the 2020 GLOBOCAN data, **bladder cancer** ranks as one of the **ten most common** cancer types throughout the world (1). Due to its high reoccurrence rate and the length of treatment, bladder cancer remains one of the most expensive cancers (2) to treat with **no significant improvements** in the standard treatment options. **Human amniotic membrane** (hAM) is an innermost fetal membrane, which is associated with a wide range of biological properties such as anti-inflammatory, anti-fibrotic and anti-microbial activity. Furthermore, recent studies have underlined the possibility that human amniotic membrane (hAM) might also act as a **promising anti-cancer agent**.

Aim

The aim of this study was to **evaluate the anticancer effect** of hAM homogenate on 2D and 3D cancer *in vitro* models.

Methods

Human muscle-invasive bladder cancer urothelial (T24) cells, papillary cancer urothelial (RT4) cells, normal porcine urothelial (NPU) cells, human mammary gland nontumorigenic (MCF10a) cells and low-metastatic breast cancer (MCF7) cells were treated with hAM homogenate. The effects of the hAM homogenate on the **desquamation of cancer cells**, **their attachment capacity, proliferation rate** and **spheroid architecture** were evaluated.

Results

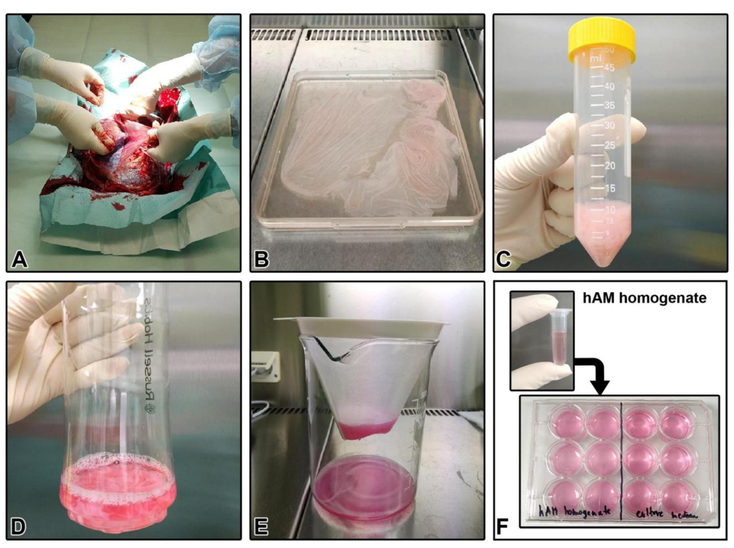

Figure 1. **Human amniotic membrane (hAM) homogenate preparation protocol.** **(A)** Separation of the hAM from human chorionic membrane (hCM). **(B)** Washing the hAM in sterile PBS. **(C)** Measuring the volume of hAM pieces. **(D)** Addition of an appropriate culture medium to the hAM pieces in the ratio of 1:4. **(E)** Filtration of hAM homogenate through sterile nylon membrane filter with pore size <1 mm, after completed homogenization. **(F)** Cryopreserved hAM homogenate is used for further experiments.

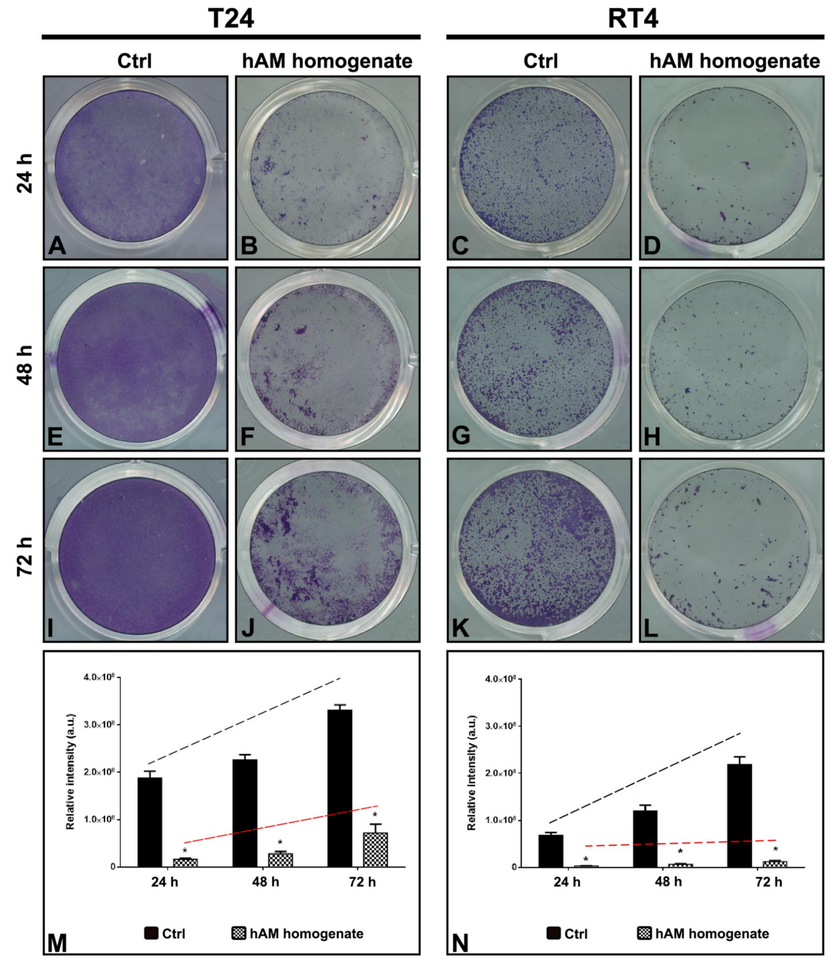

Figure 3. **hAM homogenate decreases the adhesion of T24 and RT4 cells and hinders their growth dynamics**. (A-D) hAM homogenate significantly reduced the ability of T24 and RT4 cells to attach to the culture surface after 24-h incubation. (E-L) hAM homogenate strongly inhibited the growth dynamics of the adhered T24 and RT4 cells 48- and 72-h after the cell seeding. (M,N) Quantitative analysis of the relative intensity of adherent T24 and RT4 cells. *p < 0.05.

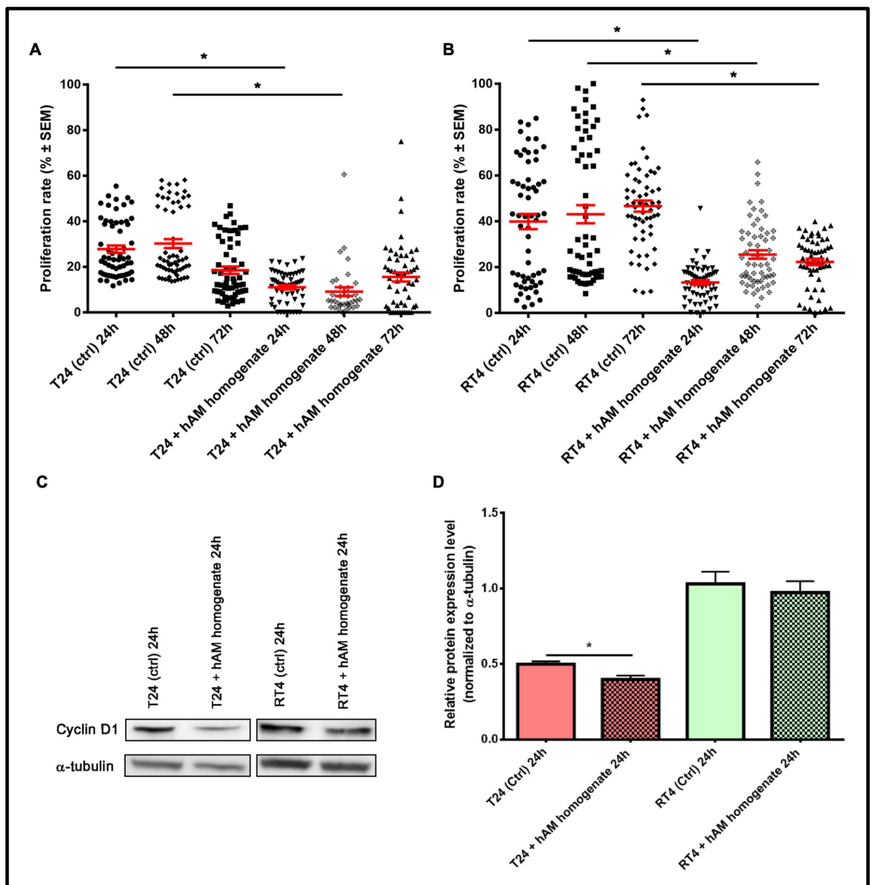

Figure 5. **Human amniotic membrane (hAM) homogenate decreases proliferation of T24 and RT4 cells and downregulates the expression of cyclin D1 in T24 cells.** (A) The proliferation of T24 cells was decreased after 24, 48, and 72 h of treatment with hAM homogenate. (B) The proliferation of RT4 cells was decreased after 24, 48, and 72 h of treatment with hAM homogenate. (C,D) The western blot analysis showed significant decrease in the expression levels of cyclin D1 after 24-h treatment with hAM homogenate in T24 cells. In RT4 cells, on the other hand, hAM homogenate induced slight but not significant decrease of cyclin D1 expression. Bars represent mean ± SEM. *p < 0.05.

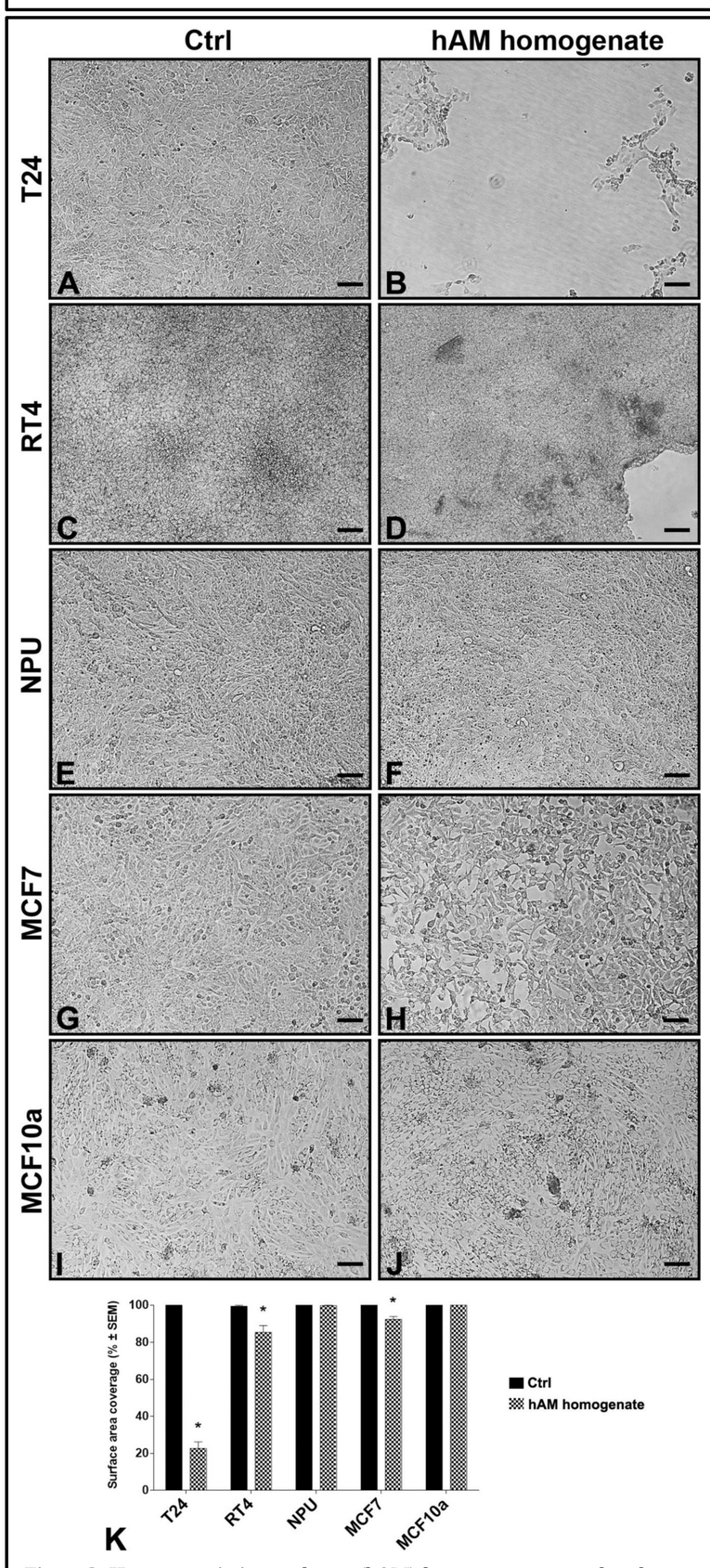

Figure 2. **Human amniotic membrane (hAM) homogenate causes detachment of various cancer cell types but not of normal cells.** (A,C,E,G,I) After 24-h incubation with an appropriate culture medium without hAM homogenate, T24, RT4, NPU, MCF7, and MCF10a cells remained firmly attached to the culture surface. (B,D,H) 24-h incubation with hAM homogenate resulted in significant detachment of cancer T24, RT4, and MCF7 cells, (F,J) but not of normal NPU and MCF10a cells. (K) The percentage of surface area covered after 24-h treatment with hAM homogenate. Scale bars: 100 µm. *p < 0.05.

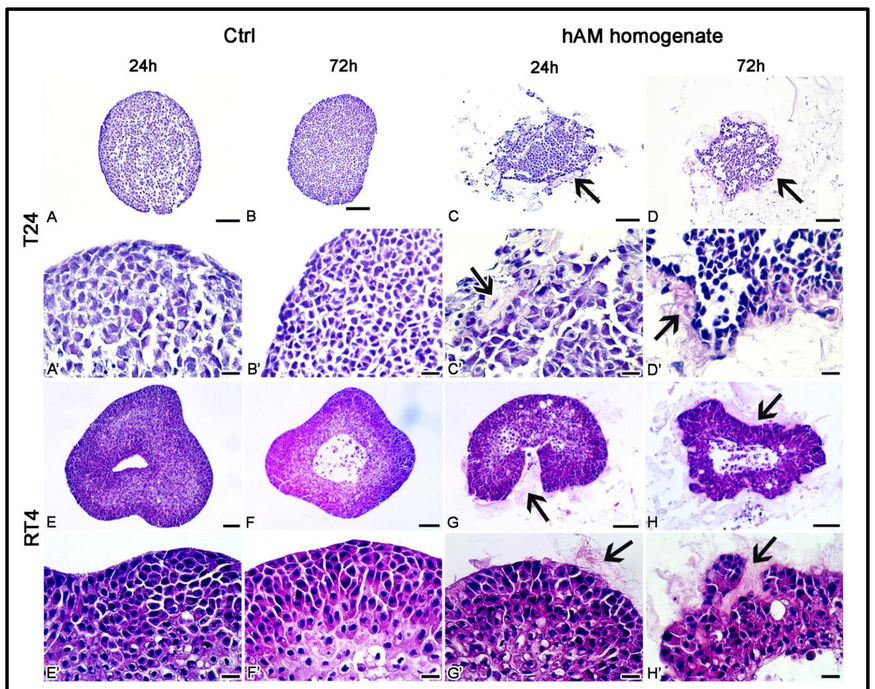

Figure 4. **Human amniotic membrane (hAM) homogenate disrupts the architecture of T24 and RT4 spheroids.** (A–B') The T24 spheroids incubated in culture medium retained a compact spherical structure. (C–D') 24- and 72-h incubations in hAM homogenate resulted in the disrupted 3D structure of T24 spheroids. hAM homogenate adhered to the surface of T24 spheroids and was in some parts even incorporated into the spheroid. (E–F') The RT4 spheroids incubated in culture medium retained a compact spherical structure. (G–H') 24- and 72-h incubations in hAM homogenate resulted in the disrupted 3D structure of RT4 spheroids as the hAM homogenate adhered to the surface of RT4 spheroids and was in some parts incorporated into the spheroid. Scale bars: (A–D, E–H) 100 µm, (A'–D', E'–H') 20 µm.

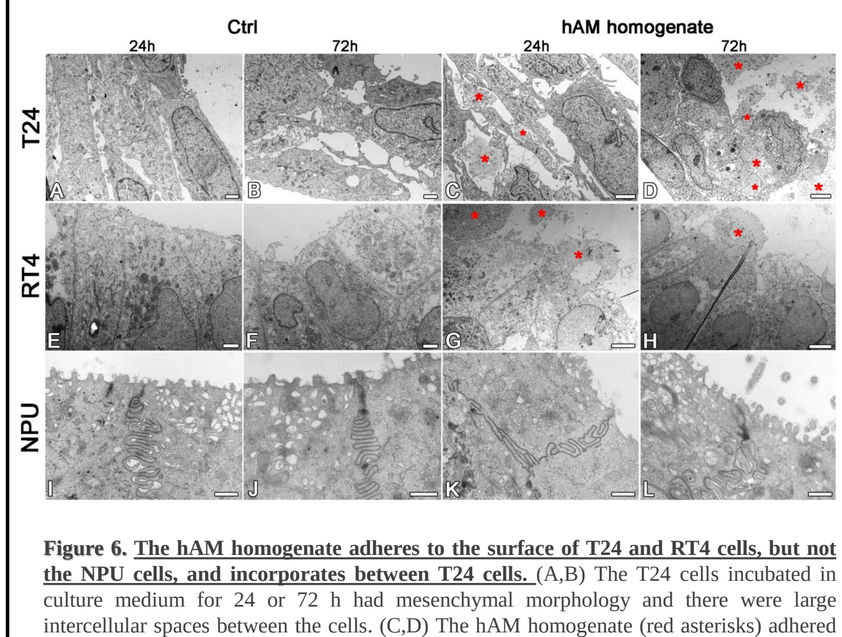

Figure 6. **The hAM homogenate adheres to the surface of T24 and RT4 cells, but not the NPU cells, and incorporates between T24 cells.** (A,B) The T24 cells incubated in culture medium for 24 or 72 h had mesenchymal morphology and there were large intercellular spaces between the cells. (C,D) The hAM homogenate (red asterisks) adhered to the surface of T24 cells and incorporated into the intercellular spaces. (E,F) The RT4 cells incubated in culture medium for 24 or 72 h had epithelial morphology and were well connected. (G,H) Incubation in hAM homogenate for 24 or 72 h had no significant effect on RT4 cell morphology. The hAM homogenate (red asterisks) adhered to the surface of RT4 cells. Some RT4 cells begin to desquamate. (I,J) The NPU cultures incubated in culture medium 24 or 72 h retained the typical ultrastructure of well-differentiated normal urothelial cells. (K,L) Incubation in hAM homogenate for 24 or 72 h had no significant effect on NPU cell morphology, and the hAM homogenate did not adhere to the surface of NPU cells. Scale bars: (A,B) 1 µm, (C) 2 µm, (D) 4 µm, (E,F) 10 µm, (G) 8 µm, (H) 6 µm, (I,L) 600 nm.

Conclusions

Human amniotic membrane has **multi-targeted anticancer activity**.

If combined with cytotoxic anticancer drugs and applied intravesically **could contribute** to bladder cancer treatment by:

- **promoting detachment** of bladder cancer cells and **preventing their re-attachment** to the urothelium,
- **decreasing proliferation** of bladder cancer cells,
- **improving targeting** of bladder cancer cells without having a toxic effect on normal urothelial cells and
- **improving drug delivery** of cytotoxic agents by **disrupting the structure** of bladder tumors (3).

References

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