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THE UROTHELIAL CELL-CULTURE RT4 EXPRESS A GLYCOSAMINOGLYCAN-LAYER ON ITS OUTER SURFACE; AN IN VITRO MODEL THAT CAN BE USED FOR RESEARCH ON THE GAG-LAYER.

Hypothesis / aims of study

The bladder urothelium contains a luminal glycosaminoglycan (GAG)-layer, also called mucin-layer, that functions as an anti-adhesive structure for pathogens and aggressive compounds found in the urine. It is vital for maintaining barrier integrity of the bladder wall. The luminal GAG-layer of the human bladder urothelium is approximately 1 cell-layer thick and consist of different proteoglycans that express three different GAG-types, respectively: heparan sulfate (HS), chondroitin sulfate (CS) and dermatan sulfate (DS).

In patients with interstitial cystitis (IC) or Painful Bladder Syndrome (PBS) who have confirmed histological abnormalities on biopsy, defects or absence of the GAG-layer is a common characteristic. This has led to the hypothesis that IC / PBS could be caused by a decreased or absent ability to construct a normal GAG-layer. A deficient GAG-layer compromises barrier integrity and leads to a leaky urothelium. Substitution of this GAG-layer with intravesical GAG replacement therapy in IC / PBS patients, uses the same principle.

Examining the characteristics of the GAG-layer has proven to be complicated. In *in vivo* experiments, the GAG-layer is easily damaged or washed away by manipulations to the bladder. It is also not easy to create an *in vivo* study design to examine the exact contribution of the GAG-layer to the total barrier function of the bladder. A representative GAG-layer grown in an *in vitro* urothelial cell culture would be far more suitable model for these tasks.

Cultured urothelial cells group together, form cell-junctions and collectively attach to the culture bottle surface. Grouped together, they grow better and survive longer. Creating a communal defensive barrier around themselves could be a causative explanation for this. Our results show that in specific cell cultures, this is done by creating a communal GAG-layer.

The aim of our study was to investigate various urothelial cancer cell cultures for their suitability as a GAG-layer-model for in vitro testing. We did this by examining their contents of HS, CS and DS and their histological appearance.

Study design, materials and methods

3 different urothelial cancer cell cultures: 2 highly differentiated (RT4 & RT122) and 1 low differentiated (Scaber), were grown at 37°C with and prepared for fluorescence immunohistochemistry and GAG-isolation. **Immunohistochemistry:** samples were blocked with goat serum and stained with antibodies for HS (HS4C3V & AO4B08V) & CS (IO3A10) and secondary with Alexa's 488 & DAPI. Specimens were evaluated by using epifluorescent confocal microscopy. **GAG-isolation & agarose electrophoresis:** Cells were harvested and treated with papain to release GAG-chains. GAG's were purified by TCA precipitation and isolated by DEAE chromatography. Isolated GAG's were isolated by agarose gel electrophoresis followed by silver staining.

Results

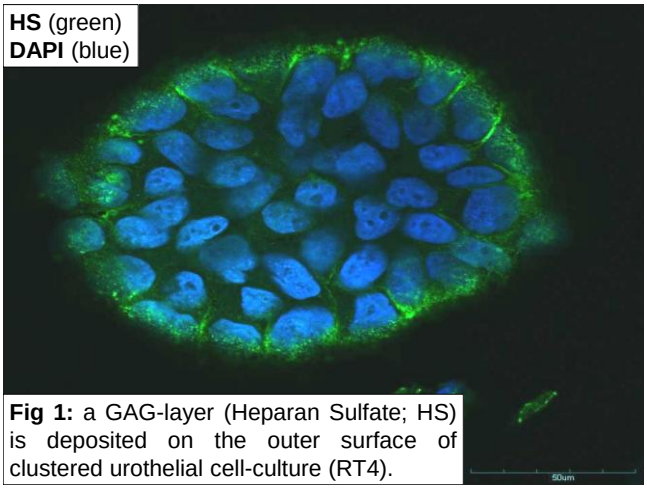

Fig 1: a GAG-layer (Heparan Sulfate; HS) is deposited on the outer surface of clustered urothelial cell-culture (RT4).

Fig 3: Electrophoresis (RT4)

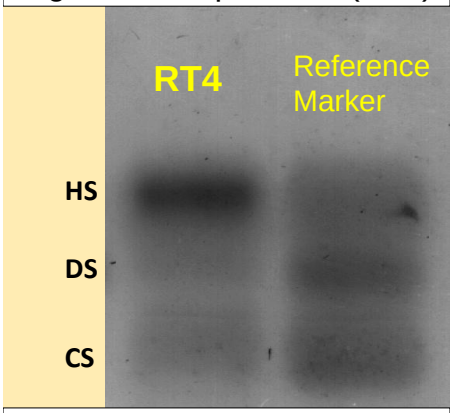

Fig 3: The RT4 urothelial cell-culture contains lots of HS, but also high amounts of CS & DS and is comparable to the luminal GAG-layer of the human bladder urothelium.

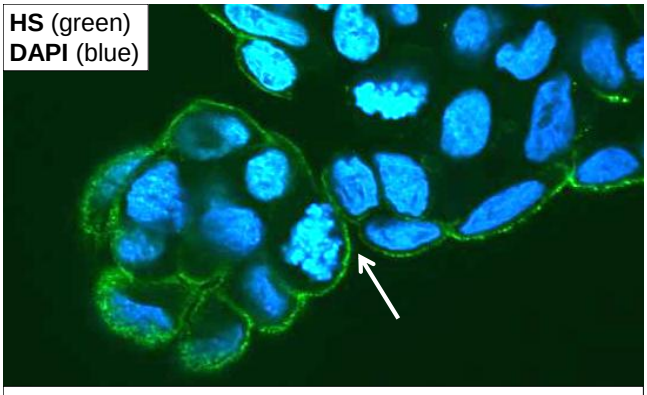

Fig 2: this detail shows two adjecent urothelial cell clusters (RT4) that each form a separate GAG-layer (Heparan Sulfate) on its outer surface (white arrow).

Interpretation of results

Of the 3 cell cultures investigated, RT4 was the only one that grew in a spherical (clustered) manner and was also the only cell culture to produce a communal GAG-layer (fig 1&2). This was confirmed in immunohistochemistry experiments with both anti-HS (fig 1&2) and anti-CS antibodies (data not shown). The GAG-isolation experiments on the RT4 cell-line showed that the GAG-layer produced by this cell culture contains HS, CS and DS in respectively decreasing concentrations (fig 3). This distribution is similar to the glycosaminoglycans that are produced by the luminal GAG-layer of the human bladder urothelium.

Concluding message

We discovered an urothelial cell culture that expresses a GAG-layer that resembles the normal luminal GAG-layer that is produced by the bladder urothelium. The well-differentiated urothelial cancer cell-culture RT4 is a suitable ***in vitro model*** that can be used to improve and facilitate research on the GAG-layer in the normal bladder and in pathological conditions like IC / PBS.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**NONE**