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Letter to the editor

The hyperchromatic supranuclear stria corresponds to the Golgi apparatus in nasal ciliated cells

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To the Editor,

The nasal mucosa of healthy subjects consists of a pseudostratified ciliated columnar epithelium, including 4 cytotypes: 1) ciliated cells, 2) muciparous goblet cells, 3) basal cells, and 4) striated cells (1). The presence of other cytotypes means acute or chronic disorders. Moreover, morphologic alterations of ciliated cells and alterations in goblet/ciliated cell ratio (normally 1:5) impair nasal physiology. Nasal cytology is commonly used in clinical practice to assess the alterations of the nasal mucosa and to evaluate changes during topical or systemic treatments (2).

Previously, it was reported the presence of a characteristic hyperchromatic area, called hyperchromatic "Supranuclear Stria" (SNS) above the nucleus of the ciliated cells (3). This hyperchromatic SNS is detected only in ciliated cells and is absent from basal, striated, and mucus-secreting cells (Figure 1A). Moreover, the high affinity of this region with basic dyes let hypothesize the presence of high protein content.

Several studies have shown that the Golgi apparatus, the smooth endoplasmic reticulum, the rough endoplasmic reticulum, and the mitochondrial organelles are in the cytoplasmic region over the nucleus of the ciliated cells (4).

Notably, chronic or recurrent inflammation affects nasal mucosa by decreasing the number of ciliated cells and increasing the muciparous goblet cells. Moreover, in the remaining ciliated cells, distress phenomena are evident and this situation determines the disappearance of the hyperchromatic SNS (Figure 1C). During respiratory viral infections, the disappearance of hyperchromatic SNS is associated with the rarefaction of the ciliary apparatus (5).

These findings led us to hypothesize that the hyperchromatic SNS existence is related to the translation of ciliary proteins and/or mechanisms of protein targeting to ciliary membranes. Therefore, we investigated the relationship between SNS and endoplasmic reticulum/Golgi apparatus, performing immunohistochemical analysis.

Smears recovered from healthy subjects showed that the ciliated cells were the most common cell type. Cells were stained for acetylated tubulin, marker of ciliated cells, and for calnexin, a marker for ER and for RCAS1, a type III transmembrane Golgi protein and more specifically localized at ER-Golgi intermediate compartment and the cis-Golgi, and for IFT20, involved in the trafficking of ciliary membrane proteins from the Golgi complex to the cilium. Additional samples of ciliated epithelium were processed for transmission electron microscope (TEM) examination. The specimens were immediately fixed in 2.5% cacodylate-buffered glutaraldehyde, pH 7.3, at 4°C for 3 hours; washed overnight in the same buffer; postfixed in buffered 1% osmium tetroxide for 1 hour; washed; dehydrated through a graded series of ethanol; cleared in propylene-oxide; and embedded in Epoxy resin (Araldite). Semithin sections cut with glass knives on an LKB V Ultratome and stained with toluidine blue

were examined by light microscope for general evaluation of tissue morphology. Ultrathin sections from selected areas were cut with a diamond knife using the same ultramicrotome, retrieved on copper grids, double-stained with uranyl acetate and lead citrate, and examined at 100 kV with a Philips 208 S electron microscope. Diagnostic electron micrographs were generally taken at a magnification of 50,000.

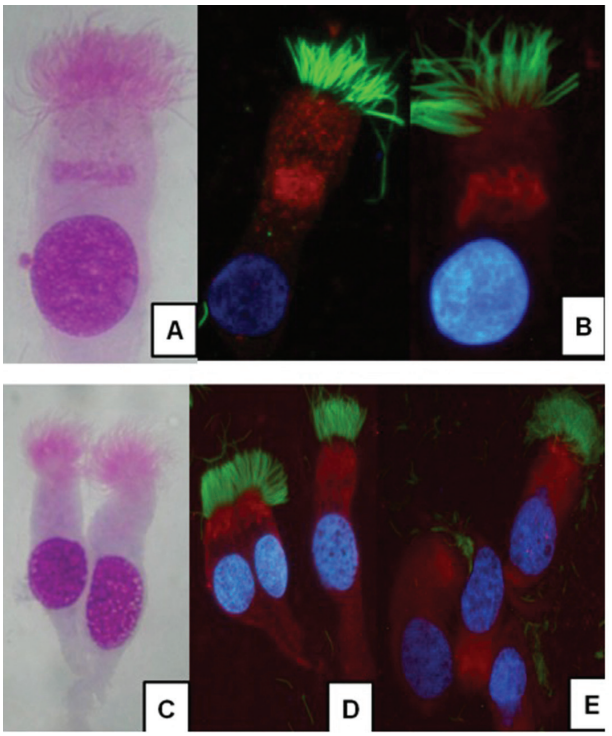

Figure 1. 1A MGG-coloured ciliated cell of nasal mucosa from a healthy subject in which the hyperchromatic SNS is evident (x1000). Figure 1C MGG-coloured ciliated cells of nasal mucosa from a patient affected by rhinitis. Representative immunofluorescence microscopy images of ciliated cells of nasal mucosa from a healthy subject (B, D) and a patient affected by rhinitis (E). Cells were fixed in 4% paraformaldehyde, permeabilized in 0.1% Triton X-100 and stained for acetylated tubulin (Figure 1B, D, E; dilution 1:300), for markers for Golgi compartment, RCAS1 (Figure 1B, left panel and Figure 1E; dilution 1:250) and IFT20 (Figure 1B, right panel; dilution 1:250) and for a marker for ER compartment, calnexin (Figure 1D; dilution 1:250). A secondary antibody conjugated to FITC (dilution 1:1000) was used to visualize acetylated tubulin (green) while a secondary antibody conjugated to TRITC (dilution 1:1000) was used to visualize RCAS1, IFT20 and calnexin (red). The visualization of nuclei (blue) was allowed by DAPI (4',6-diamidino-2-phenylindole) staining

Figures 1D shows that calnexin was diffusely distributed in cytoplasm of ciliated cells, while both markers for Golgi compartment, RCAS1 (Figure 1B, left panel) and IFT20 (Figure 1B, right panel), were localized in a restricted area amidst the nucleus and cilia, the same region of hyperchromatic SNS previously observed by immunohistochemical analysis (3). Interestingly, ciliated cells of patients affected by viral and allergic rhinitis showed RCAS1 signal uniformly distributed in the cytoplasm, confirming the absence of SNS (Figure 1E).

Figure 2 shows the identification of the Stria by the Transmission electronic microscopy (TEM).

Moreover, our results, supported by the literature findings of previous studies (5,6), put in evidence the loss of cilia in ciliated cells derived from patients affected by rhinitis.

We speculate that the SNS is constituted by protein components of the ciliary apparatus. In patients affected by viral and allergic rhinitis the rarefaction of the SNS, probably consequent to a reduction of protein synthesis and/or to down-regulation of protein transcription, associated to the dispersion of Golgi elements, corresponded to a loss of cilia. This finding suggests a close relationship between SNS and cilia mediated by the Golgi apparatus.

Also, the current study confirmed that Stria (SIS) places in the Golgi apparatus, as provided by immuno-

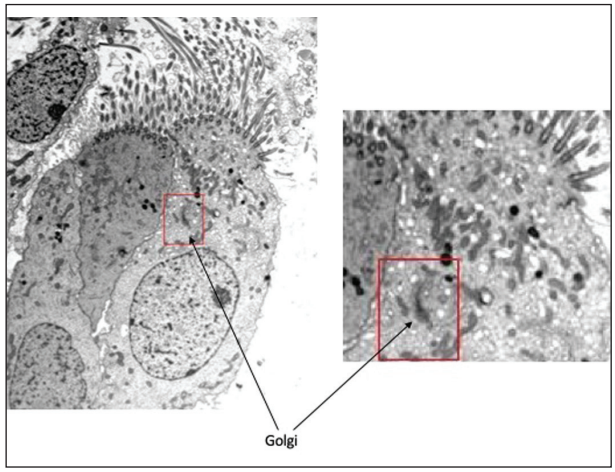

Figure 2. Median sagittal section of ciliated cells with localization of Golgi apparatus by TEM (Transmission electronic microscopy), magnification x 89.000

histochemical and electronic findings. The presence (or absence) of Stria is associated with ciliated cell activity and wellness. In particular, in our experience Stria is present essentially in the large majority of the normal epithelial cells, whereas it disappears in subject with nasal disorders, including infections and inflammatory diseases. The molecular mechanisms that justify the presence of the Stria are related to the physiological biochemical metabolism. In fact, during infection and/or inflammation it disappears.

Therefore, thanks to these up-to-dated techniques, it was possible to detect the real nature of the hyperchromatic SNS in an incontrovertible way.

In conclusion, there is evidence that the Hyperchromatic Supranuclear Stria corresponds (colocalizes) to the Golgi apparatus and its presence defines the wellness of ciliated cells. Moreover, it will be important to analyze the mechanism that induces to the rarefaction of the stria. In this light, deeper studies will be performed.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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