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Exfoliated epithelial cells: a source of biological information in preterm infants.

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Introduction

Exfoliated epithelial cells can be isolated from digestive fluids as a cellular mixture (senescent, apoptotic, proliferative, or quiescent cells). By quantifying the degree of exfoliation, i.e. by assessing the loss of epithelial cells or of their specific molecular compounds, it is possible to evaluate pharmacological or nutritional stress in clinical trials.

Here we search for the expression of NPAS2, a CLOCK isoform & the expression of biomarkers of stem cell and of neuroendocrine lineage.

Material & Methods

Written informed consents were obtained from both parents to collect gastric fluid aspirates of 21 infants (Biocollection PROG/09/18).

We used a single procedure to isolate epithelial cells from gastric fluid aspirates (1) or stools (2) of preterm infants.

Here gastric fluids were:

- (1) Resuspended (10 cycles) in 10 ml Buffer (1 mM EDTA / 0.05 mM DTT in PBS0)
- (2) Centrifuged at 1300 rpm, 5 min, RT, to pellet cells
- (3) Rinsed in PBS0 and centrifuged
- (4) Cell pellet is either fixed for microscopy or stored at -70°C

Fixed cells were immunolabeled & observed by confocal imaging (1)

Stored cells were lysed for qRT-PCR (2)

Conclusions

- (1) Along with previously described PERIOD1 & CLOCK (1), we show that NPAS2, an isoform of CLOCK, is expressed in parietal cells of preterm infants as reported in mouse stomach (3). NPAS2 is critical for adaptability to food restriction (7)
- (2) Adaptation of primary cultured cells to a 3D long-term culture system (4) may be used to select epithelial cells from the neuroendocrine lineage.

Perspectives

Transcriptomic analyses have been done on epithelial cells recovered from gastric fluid aspirates (2) and stools (2, 5, 6).

We intend to design a system allowing the co-detection of messenger RNA and microRNA to evaluate the physiological state of infants.

By taking advantage of the Triocapi cohort (NCT 0163 0278), gastric fluid aspirates of preterm infants receiving different Ibuprofen doses, integrative miRNA-mRNA profiling will be used to evaluate the degree of gastric exfoliation.

Results

Figure 1. NPAS2 expression in gastric exfoliated cells.

Cells were recovered from infants between Day 5 to Day-36 after birth (A, D; E: Background) and labeled for nuclei by Hoechst (blue head arrow) and NPAS2 (red head arrow, Santa Cruz, sc28708).

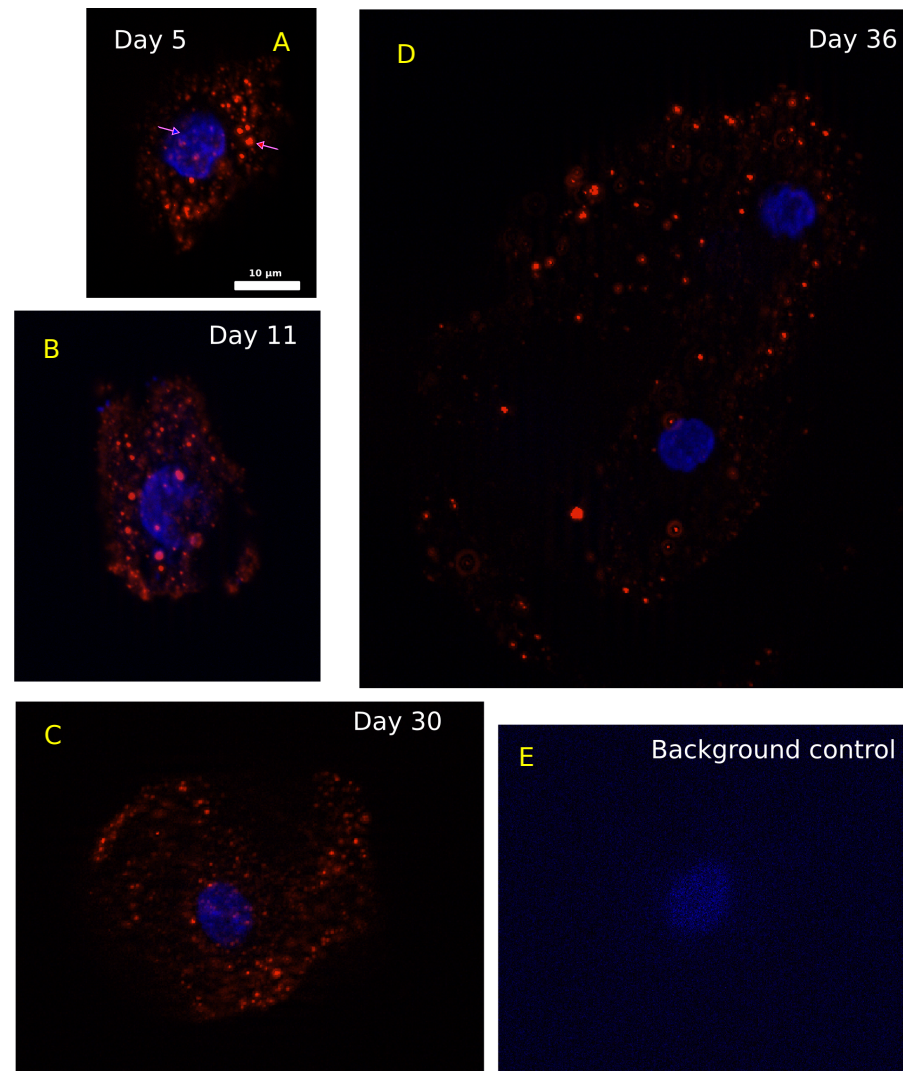


Figure 2. LGR5 & Chromogranin A expressions by gastric cells freshly isolated (A, B) or after 16 days of primary culture (C).

A. Membrane labeling of LGR5 (red head arrow; Santa Cruz sc-68580) of a typical epithelial cell (nucleus in blue, blue arrow head).

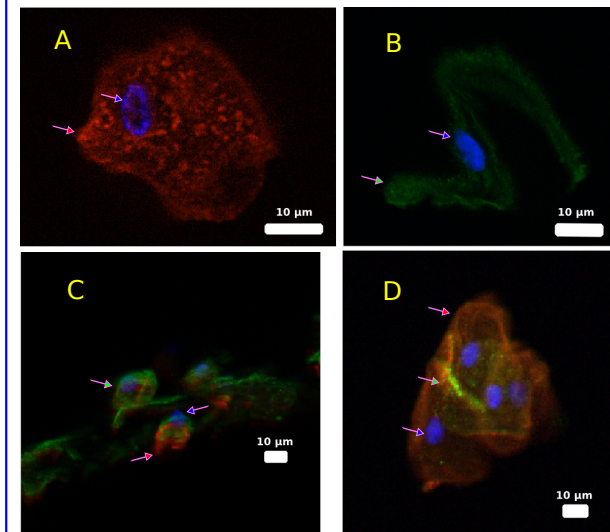
B. Chromogranin-A-positive cell (green head arrow, Spring Bioscience E1520). Nucleus in blue (blue arrow head).

No detection of positive cells for ngn3 biomarkers on isolated exfoliated cells except by qRT-PCR (unshown). Analyses were performed on gastric fluid aspirates of 7 infants. Cells positively labeled for LGR5 & Chromogranin A were found on 2 samples.

C. Chromogranin-A-positive cells (Chromogranin A; red arrow head; H+/K+ ATPase, Abcam, ab-2866; green arrow) in primary culture.

D. Some cell colonies with typical epithelial morphology were co-expressing Oct-4 (Abcam, ab-49091; red head arrow), H+/K+ ATPase (green head arrow), confirming previous results (1).

On gastric aspirates of 5 infants, we had 2 primary cultures with Oct-4-positive cells. Only a single colony was labeled by antibody against chromogranin A.



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