

 <p>UniSR Università Vita-Salute San Raffaele</p>	<p>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</p>	<p>MO 20-5 ed. 01 del 21/02/2025 PO 20 Page 4 of 10</p>
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PROJECT The role of Anterior Gradient Homolog 2 (AGR2) and Endoplasmic Reticulum Protein-Retaining Receptor (KDEL3) in the pathophysiology of Barrett's Esophagus: from mechanisms to clinical implications.

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Title: MD, PhD

Curriculum: Clinical and Experimental Medicine

Link to the personal page of the University or relevant hospital site website: <https://www.unisr.it/docenti/d/danese-silvio>

Description of the Project (max 3,000 characters including spaces)

Background. Barrett's esophagus (BE) is a rare disease (ORPHA:231080) where the distal esophageal squamous mucosa is replaced by the metaplastic columnar mucosa (specialized intestinal metaplasia, SIM), a precancerous lesion containing intestinal-like cells such as goblet cells (GCs). Gastroesophageal-reflux disease (GERD) and genetic susceptibility are the main risk factors. BE patients, due to the SIM-dysplasia sequence, have a risk of up to 55 times of developing esophageal adenocarcinoma (EAC), with a continuously rising incidence. In this view, an urgent need to better understand BE pathophysiology arises, to enhance prevention and treatment. An intriguing field lies in understanding the mechanisms and factors involved in the squamous epithelium's SIM transdifferentiation/transcommitment. GERD stimulates GCs to produce mucin proteins, neutralizing acidity, and thus promoting the SIM process. Anterior Gradient Homolog 2 (AGR2), a disulfide isomerase protein responsible for protein folding in the endoplasmic reticulum (ER) and mucin secretion by GCs, was associated with BE pathogenesis. The proper maturation of mucins, including the GCs marker Mucin 2 (MUC2), also relies on AGR2, which binds to the ER lumen protein-retaining receptors (KDEL3s) in the Golgi. Indeed, the AGR2 loss of function compromises not only MUC2 secretion but also the terminal differentiation of GCs. **Rationale/hypothesis.** Existing evidence has demonstrated that the AGR2 expression is directly correlated with the BE-specific mucin expression in BE samples, where it is up-regulated compared to healthy controls. Furthermore, KDEL3 is the only receptor in the KDEL3 family that is overexpressed, showing a positive correlation with AGR2 in BE samples. Based on this evidence, we hypothesize that KDEL3, a potential cognate factor in the AGR2-directed mucin release in GCs, orchestrates, with/without AGR2, the BE metaplastic events and that its specific deletion may contribute to reverting the squamous-to-columnar transition. **Objectives/specific aims.**



1) To verify the KDEL3 functions in directing the SIM process of the esophageal epithelium in vitro;
2) To assess the KDEL3 deletion as a possible intervention for reverting the squamous-to-columnar transition in BE patient-derived cells; 3) To assess the AGR2/KDEL3 role in the BE disease history. **Expected outcomes.** To date, the mechanisms underlying BE metaplasia have not been entirely defined, and a deeper understanding could have significant implications for EAC prevention and BE treatment. Our proposal aims to study and clarify BE pathogenesis and GCs fate commitment mechanisms (SIM transdifferentiation) in which both AGR2 and KDEL3, by promoting mucin production/release, could play a crucial role. From a translational perspective, during our patient-focused study, we aim to investigate whether inhibiting these factors in BE patient-derived organoids can halt transdifferentiation and thus represent a potential new targeted therapy for BE patients, whose current treatments are limited.

Skills that the student should acquire (max. 600 characters including spaces):

- Primary cell derivation from mucosal esophageal biopsies;
- Molecular and cellular biology techniques;
- Familiarity with computational analysis.

References (max. 15)

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7. Que, J., Garman, K. S., Souza, R. F. & Spechler, S. J. Pathogenesis and cells of origin of barrett's esophagus. *Gastroenterology* 157, 349–364.e1 (2019).
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9. Park, S.-W. et al. The protein disulfide isomerase AGR2 is essential for production of intestinal mucus. *Proc Natl Acad Sci USA* 106, 6950–6955 (2009).
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12. Zhang, H. et al. Intracellular AGR2 transduces PGE2 stimuli to promote epithelial-mesenchymal transition and metastasis of colorectal cancer. *Cancer Lett.* 518, 180–195 (2021).
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