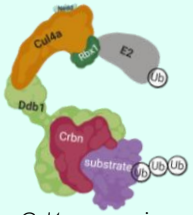
CULLIN4 REGULATES COLORECTAL CANCER EXPANSION THROUGH THE MODULATION OF INTRACELLULAR SMAD3 TRAFFICKING

Iatsiuk Veronika[1], Tureckova Jolana[1], Prochazkova Michaela[1], Michalcikova Tereza[1], Spoutil Frantisek[1], Talacko Pavel[2], Novosadova Vendula[1], Sedlacek Radislav[1], Prochazka Jan[1]

[1]*Laboratory of Transgenic Models of Diseases and Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*BIOCEV proteomics core facility, Faculty of Science, BIOCEV, Charles University, Vestec, Czech Republic*

Background

Colorectal cancer (CRC) is a second leading cause of cancer-associated mortality. Among others, cancer development is associated with alteration in ubiquitination process.



Cullin4-RING E3 ubiquitin ligase (CRL4) is an important complex, responsible for regulation of signaling pathways. The core component is CUL4A that works as a scaffold by assembling the multicomponent CRL4.

Aberrant expression of the *Cul4a* gene is a associated with many tumor types, including CRC.

Thereby, **in this study we aim to identify the role of CRL4 in the alteration of the regulatory pathways, resulting in the gastrointestinal homeostasis disorders and tumor expansion.**

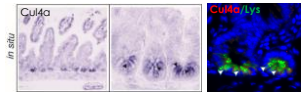
Methods

- Cul4a tm1b mice were used as a model for characterizing GIT physiology in Cul4a knockout.

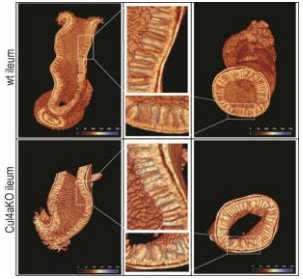
- To assess the role of Cul4a in the CRC initiation and progression, we were using CRC mouse model – Apc$^{Min/+}$.
- For identification of Cul4a interaction partners we used a combination of pull-down assay, mass spectrometry analysis and co-IP, followed by siRNA down-regulation in CRC cell line together with IHC and IF staining.

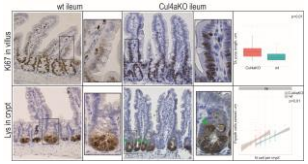
Results

I. Culin4a is expressed by Paneth cells in the crypt base and its knockout promotes development of anomalies in GIT



- Colocalized expression of Cul4a and Lys = Paneth cells are Cul4a expressing cells
- PCs produce regulation factors for stem sells and have stem-like features





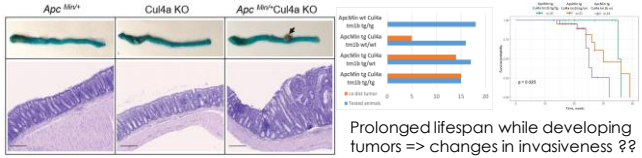
I. Cul4aKO give rise to misshapen villus morphology in small intestine (SI), but
- NO tumor development
- NO critical physiological effects

II. Enlargement of proliferative zone and abnormal localization of Lys+ cells in the crypt base was observed in Cul4aKO mice

II. Cul4KO on *Apc$^{Min/+}$* background leads to tumor development in distal colon

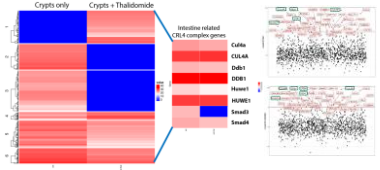
- Min = multiply intestinal neoplasia
- Apc$^{Min/+}$ mice developing multiple adenomas in SI

- All tested Apc$^{Min/+}$Cul4aKO develops tumors in SI and colon
- Tumors is strictly located in the distal part of the colon

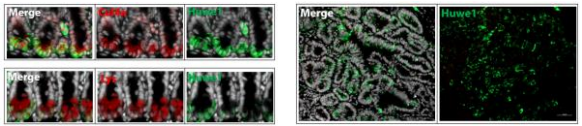


Prolonged lifespan while developing tumors => changes in invasiveness ??

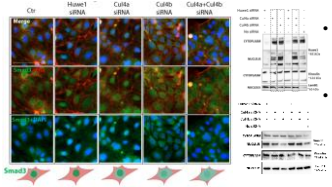
III. Huwe1 and Smad3 are most probable Cul4a interaction partners in the intestinal tumorigenesis



Pull down experiment with CRBN-tag detects:
- CRL4 components: Ddb1, Cul4a/b, Crbn
- Huwe1 – HECT E3 Ub ligase
- Smad3 – modulator of TGF-β signaling



- Huwe1 expression is colocalized with Cul4a and Lys in the crypt base
- Enrichment of Huwe1 expressing cells in SI and colon tumors



- Downregulation of Cul4a and Huwe1 affects cytoplasmic : nuclear trafficking balance of Smad3
- Cul4a and Cul4b working as negative regulators of Huwe1:

Cul4a ⊣ Cytoplasmic Huwe1

Cul4b ⊣ Nuclear Huwe1

Conclusions

Our results show that Cul4a is a critical regulatory element in the intestine homeostasis that associated with colorectal cancer progression and invasiveness.

Cul4a is likely influencing Smad3 intracellular trafficking through the negative regulation of Huwe1 that works as ubiquitination agent for Smad3. This could be a probable mechanism of tumor severity and its development in the distal colon.

However, the role of Cul4a in the tumor invasiveness needs to be clarified.

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education
MINISTRY OF EDUCATION, YOUTH AND SPORTS
IMG
Czech Centre for Phenogenomics
BIOCEV

Financial support was provided by the Czech Academy of Sciences (RVO 68378050), by MEYS (LM2018126 and LQ16014), by MEYS & ESIF CZ.02.1.01/0.0/0.0/16_013/0001789 Upgrade of the Czech Centre for Phenogenomics: developing towards translation research and CZ.02.1.01/0.0/0.0/18_046/0015861 CCP Infrastructure Upgrade II
Keeping sustainability of OP RDI (CZ.1.05/1.1.00/02.0109 'Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University in Vestec (BIOCEV) & CZ.1.05/2.1.00/19.0395 - Higher quality and capacity for transgenic models breeding) by MEYS and ERDF
The funders had no role in study design, data collection, analysis, decision to publish, or preparation of the poster.