Proteomic Analysis of Barrett's Esophagus Cells

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Introduction

- Barrett's esophagus (BE) is a disorder in which the normal squamous mucosa in the esophagus is replaced by metaplastic columnar epithelium.
- BE occurs in 15% of patients with gastroesophageal reflux disease and 1-2% of the overall adult population. It is the highest risk for esophageal adenocarcinoma (EAC) and patients with BE have at least 10 times greater risk of developing EAC.
- Large sample amounts are typically required to achieve deep proteomic coverage and sample loss may occur during sample preparation steps, limiting the analysis of small sample amounts.
- Recently, the Microdroplet Processing in One pot for Trace Samples (µPOTS) platform was developed for proteomic analysis of small cell populations.[1] Bottom-up proteomic sample preparation occurs in microwells with volumes of 2 µL, thereby reducing adsorptive protein losses.
- Non-dysplastic BE cell lines (CPA) p53 KO and p53 Smad4 KO cells were generated with the CRISPR/Cas9 genome-editing technology and stimulated with lithocholic acid (LCA) or X-ray.
- **Goal:** determine differentially expressed proteins between CPA WT, CPA p53 KO and CPA p53 KO Smad4 KO treated with LCA or X-ray by performing bottom-up proteomic sample preparation with samples containing 200 cells using the µPOTS platform.

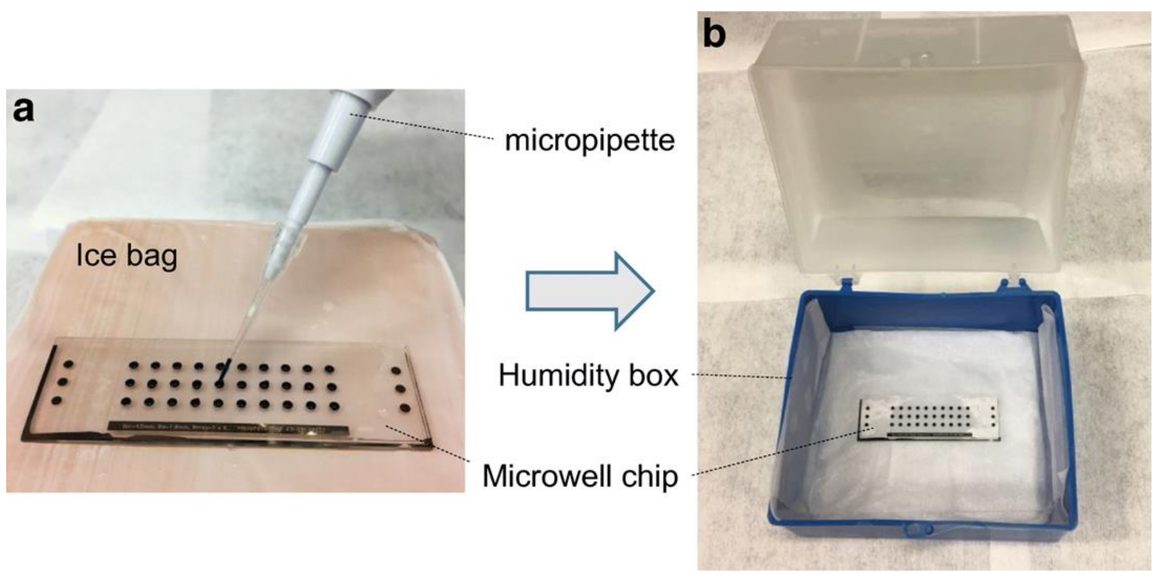

Figure 1. Microwell chip containing wells with diameters of 2 mm is placed on an ice bag to prevent sample evaporation during addition of reagents **(a)** and in a humidity box during incubation **(b)**.[1]

Methods

Samples containing 200 cells

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Protein extraction and reduction with 0.1% DDM and 50 mM DTT in microwells

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Alkylation with 10 mM IAA

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Trypsin digestion

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nanoLC-MS/MS analysis on an Orbitrap Lumos instrument

Results

Protein extraction from 200 cells with µPOTS strategy

- More than 1500 protein groups were identified with CPA samples containing 200 cells.

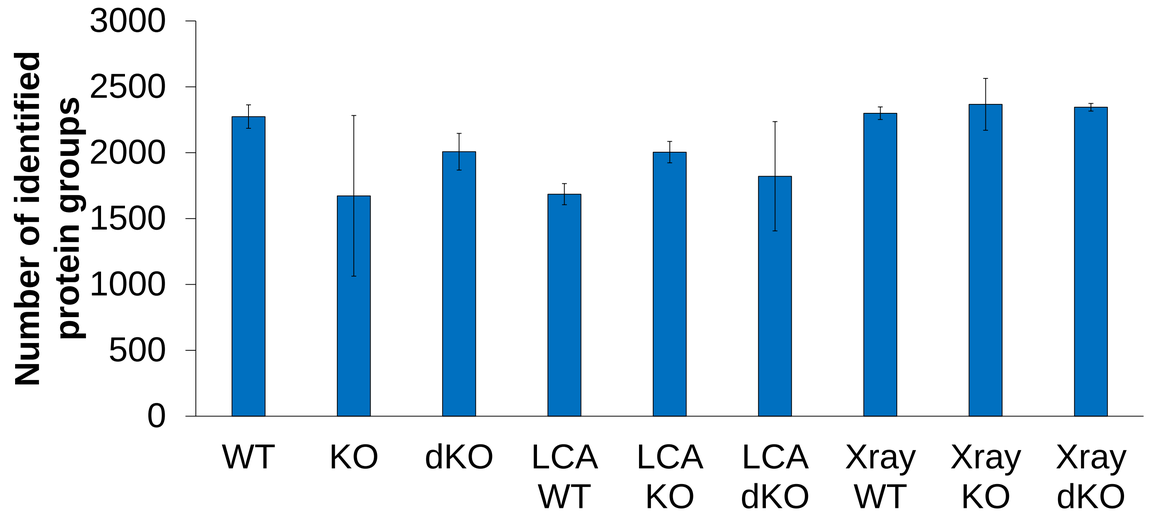

Figure 2. Number of identified protein groups from samples containing 200 cells with the µPOTS strategy.
KO: p53 KO, dKO: p53 KO Smad4 KO

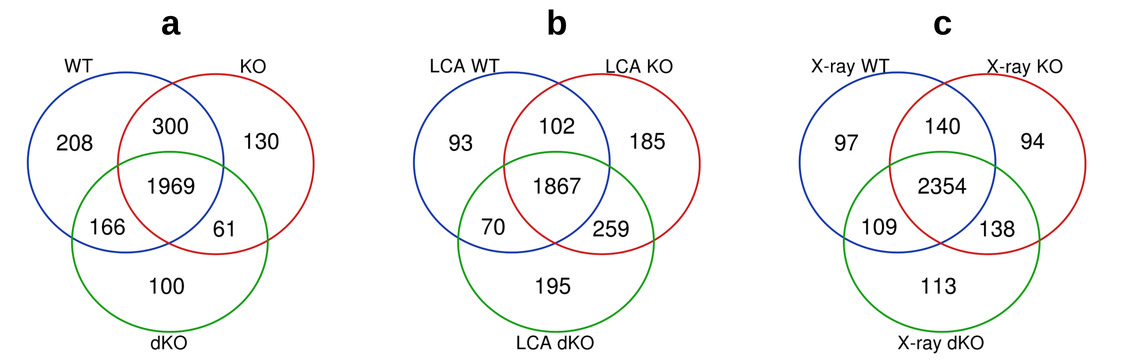

Figure 3. Protein overlap between WT, KO and dKO **(a)**, LCA WT, LCA KO and LCA dKO **(b)** and X-ray WT, X-ray KO and X-ray dKO **(c)**.

- N-myc downstream-regulated gene 1 (NDRG1) was found in WT when comparing WT vs LCA WT, in LCA KO when comparing LCA WT and LCA KO, in X-ray WT when comparing LCA WT and X-ray WT and in X-ray dKO when comparing dKO vs X-ray dKO.
- NDRG1 has been reported to suppress tumor growth and metastasis.[2]

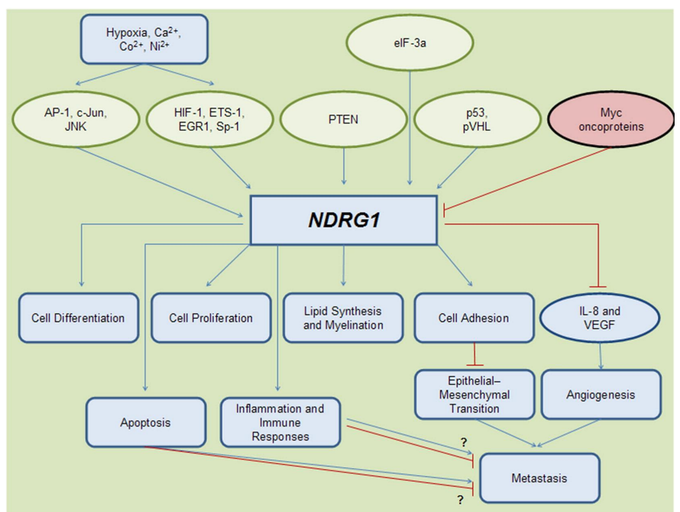

Figure 4. Biological functions and regulation of NDRG1.[2]

Conclusions

- The high sensitivity of the µPOTS platform was demonstrated by analyzing samples containing 200 cells.
- NDRG1 was shown to be a major responder in different cell lines with different stresses.

References

1. Xu, K., et al. Anal. Bioanal. Chem. (2018)
2. Fang, B. A., et al. Biochimica et Biophysica Acta (2014)

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