FINAL REPORT (K 109626)

Project title: Investigating the apelin/APJ pathway in chest tumors

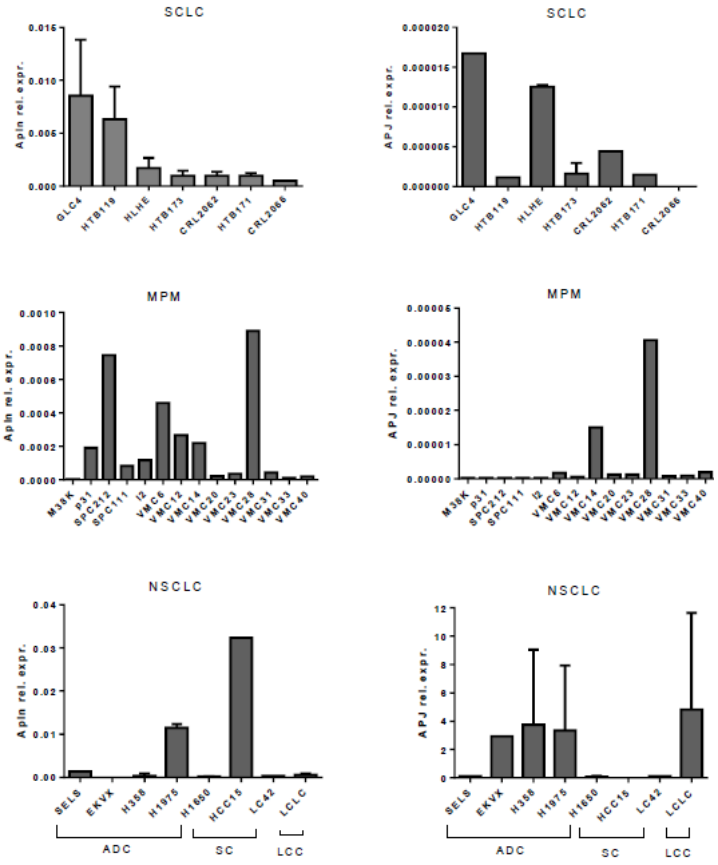
Home Institute: National Koranyi Institute of Pulmonology, Budapest, Hungary

Rationale and Aim

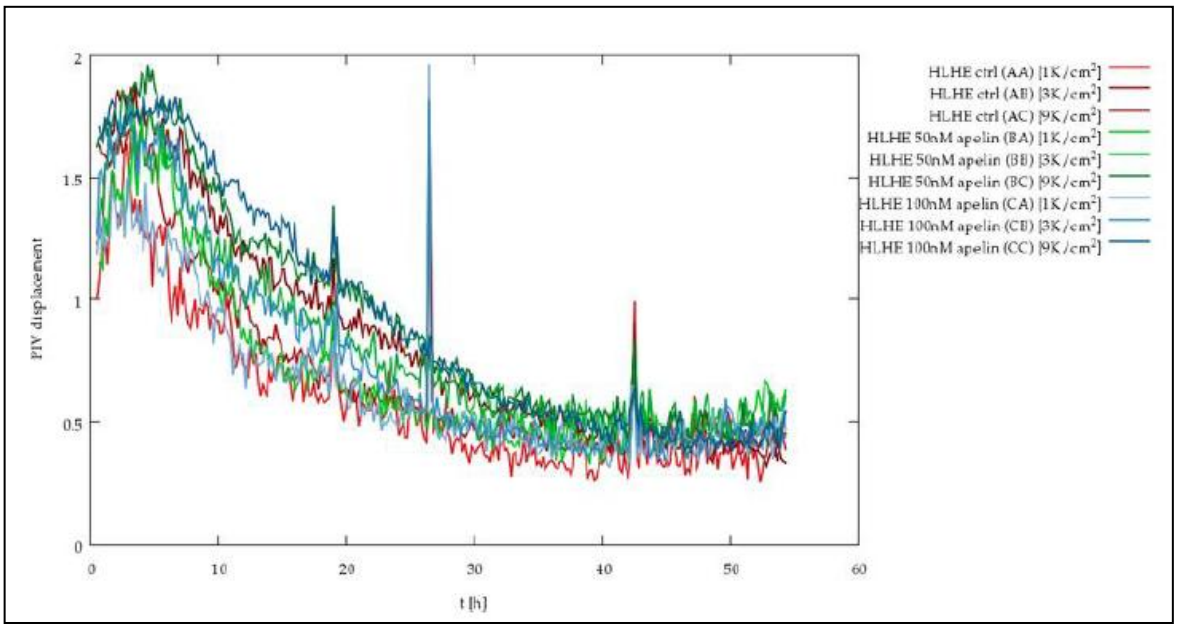
This project aimed to investigate the biological and clinical significance of apelin in the growth of lung cancers and mesothelioma.

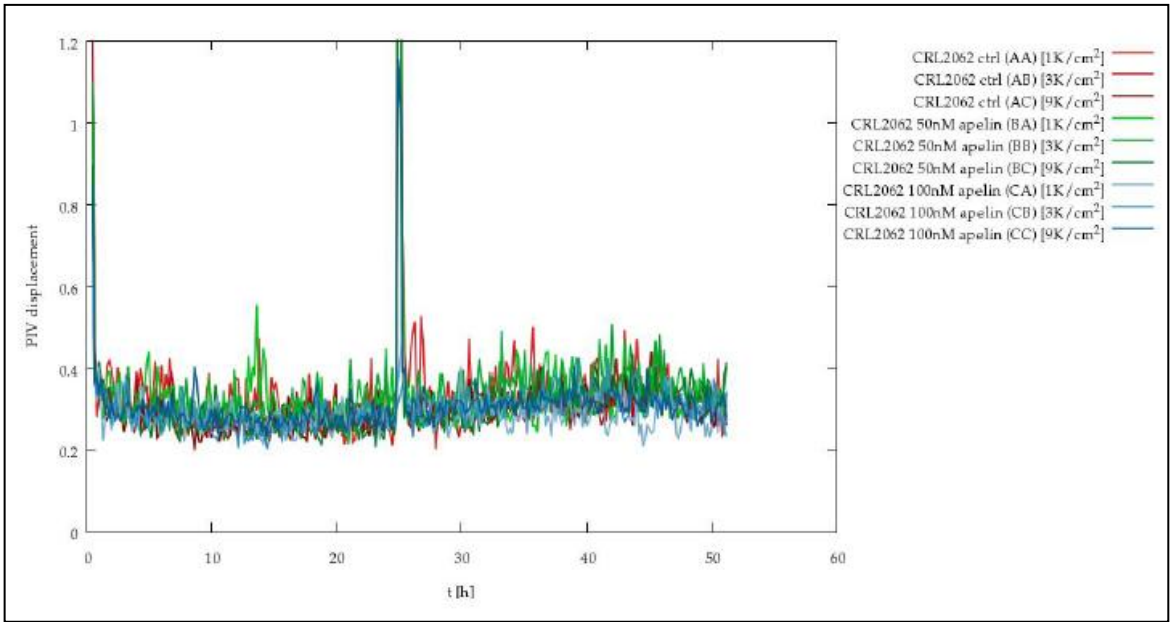
In vitro results

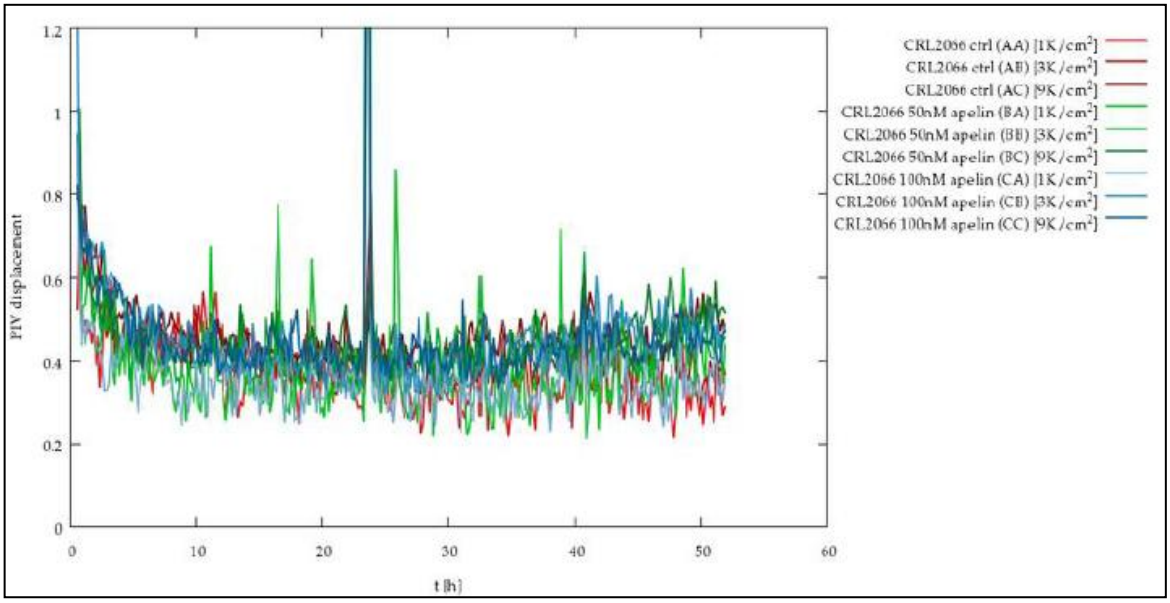
As proposed in our original research plan, we further analyzed the apelin and apelin receptor (APJ) expression profiles of our small cell lung cancer (SCLC), malignant pleural mesothelioma (MPM) and non-small cell lung cancer (NSCLC) cell lines (including ADC: adenocarcinoma; SC: squamous cell carcinoma; LCC: large cell carcinoma cells). The different apelin/APJ mRNA expressions are shown below.



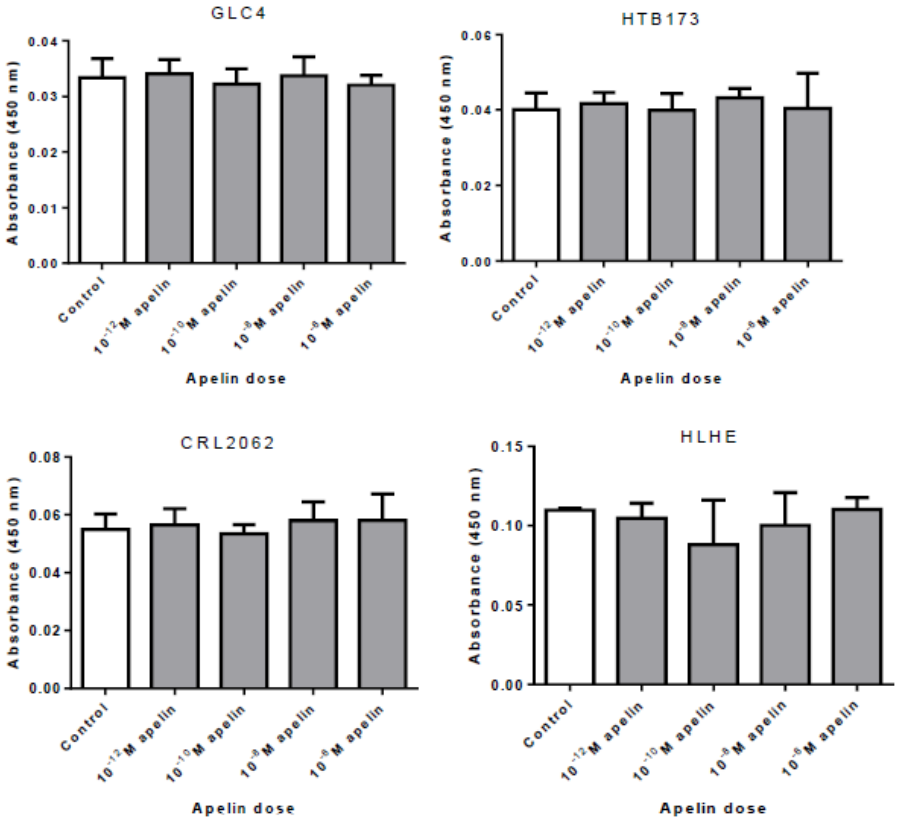
Next, we treated three adherent SCLC cell lines (HLHE; CRL2062; CRL2066) with 50 nM or 100 nM apelin-13 (Phoenix Pharmaceutical; Burlingame, CA, USA) to study whether exogenous apelin treatment influences the migratory activity of SCLC cells at different cell densities (90 vs. 30 vs. 10 cells/mm2). However, as shown below, apelin did not exhibit a significant pro- or anti-migratory effect on these the cells.


PIV displacement
t [h]
HLHE ctrl (AA) [1K/cm²]
HLHE ctrl (AB) [3K/cm²]
HLHE ctrl (AC) [9K/cm²]
HLHE 50nM apelin (BA) [1K/cm²]
HLHE 50nM apelin (BB) [3K/cm²]
HLHE 50nM apelin (BC) [9K/cm²]
HLHE 100nM apelin (CA) [1K/cm²]
HLHE 100nM apelin (CB) [3K/cm²]
HLHE 100nM apelin (CC) [9K/cm²]


PIV displacement
t [h]
CRL2062 ctrl (AA) [1K/cm²]
CRL2062 ctrl (AB) [3K/cm²]
CRL2062 ctrl (AC) [9K/cm²]
CRL2062 50nM apelin (BA) [1K/cm²]
CRL2062 50nM apelin (BB) [3K/cm²]
CRL2062 50nM apelin (BC) [9K/cm²]
CRL2062 100nM apelin (CA) [1K/cm²]
CRL2062 100nM apelin (CB) [3K/cm²]
CRL2062 100nM apelin (CC) [9K/cm²]

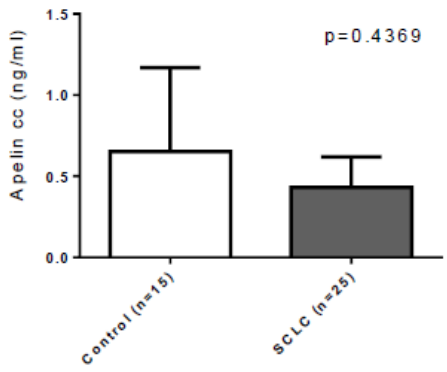


In line with the migration data and as shown in the below figure, SCLC cells treated with exogenous apelin at different concentrations did not show significantly altered proliferation activity.



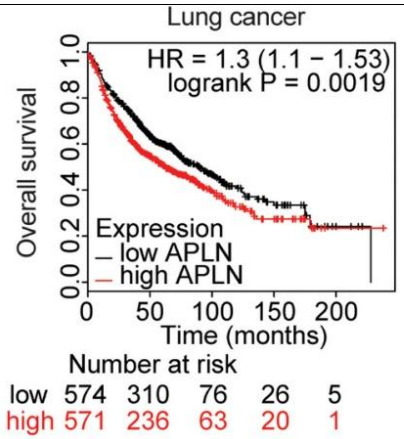
Human studies

In our experiments using human blood samples, we found a slight -but statistically not significant decrease - in the circulating levels of apelin (as measured by ELISA) in patients with SCLC (n=25) vs. tumor-free individuals (n=15). p=0.4369

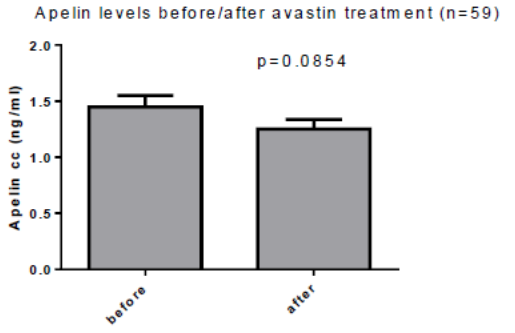


By performing a Kaplan–Meier analysis from the KM-plotter database (Gyorffy et al. PLoS One 2013;8:e82241) for high and low apelin-expressing groups in lung cancer patients (see the attached graph), we could confirm our previous results (Berta J et al. J Thorac Oncol 2010;5:1120-9.) that high levels of apelin expression are significantly associated with poor prognosis in NSCLC. For details, please refer to our recent publication: Uribesalgo I, et al. EMBO Mol Med 2019;11:e9266. Importantly, in this collaborative study we report that combining apelin inhibition with the antiangiogenic drug sunitinib markedly reduce tumor growth and increases survival in animal models of lung cancer.

It is also important to mention that in the aforementioned study (Uribesalgo I, et al. EMBO Mol Med 2019;11:e9266) we found that apelin levels in renal cancer patients receiving sunitinib (an anti-angiogenic drug) correlated with worse survival. These data also suggest that blood apelin levels in sunitinib-treated renal cancer patients may represent a potential biomarker to predict the efficacy of anti-vascular drugs and to identify patients responsive to these therapies.



Notably, however, we did not found a significant difference in the apelin plasma levels of patients with NSCLC (n=59) before and after bevacizumab treatment. We also showed that high or low apelin levels did not have an effect on the progression-free survival (PFS) of these patients receiving bevacizumab (Avastin). For details, please see the below figures.



	Before			After		
	Mean	SD	Range (ng/ml)	Mean	SD	Range (ng/ml)
Apelin cc.	1.448	0.7959	0.3364-3.879	1.252	0.6538	0.4759-3.525