Bone & Joint Research

Supplementary Material

10.1302/2046-3758.105.BJR-2020-0262.R3

Table i. G protein subunit alpha Q (GNAQ) status in patient-derived cancer cells (PDCs) from patients with primary lung cancer and bone metastasis of lung cancer. We used a CancerSCAN panel that can identify mutations, deletions, and amplifications for 381 cancer-related genes.

Variable	Number of samples	Number of samples with mutations in GNAQ (%)
Primary		
Lung cancer	53	0 (0)
Bone metastasis		
Lung cancer/Non-small cell lung cancer	27	11 (40.7)

GNAQ, G protein subunit alpha Q.

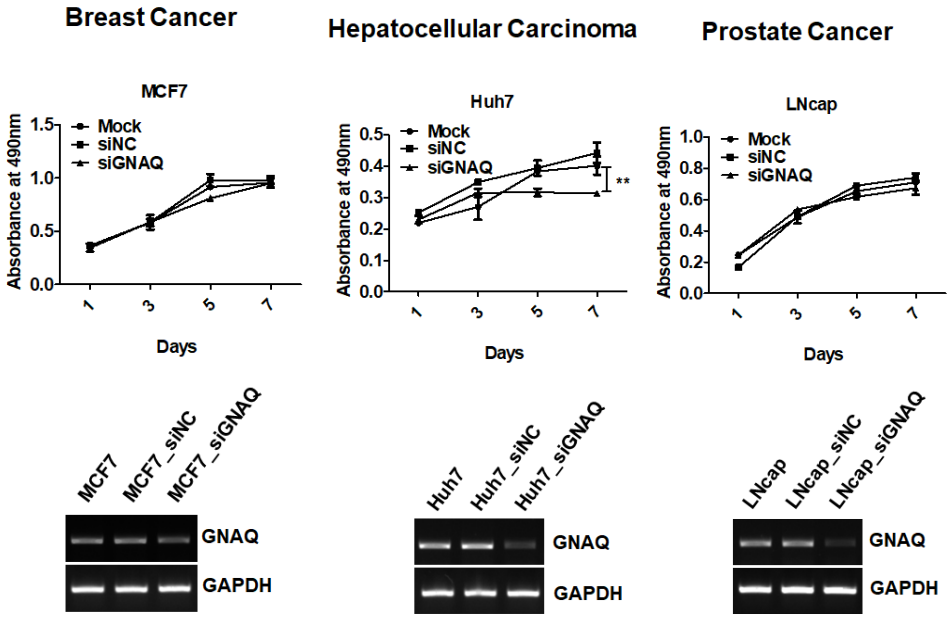

Fig. a. The effect of G protein subunit alpha Q (GNAQ)-knockdown on the proliferation of various types of cancer cells. *GNAQ* small interfering RNA (siRNA) was transfected into a breast cancer cell

line (MCF7), hepatoma cell line (Huh7), and prostate cancer cell line (LNcap), with negative siRNA serving as a control. GNAQ-knockdown inhibited cell proliferation in various cancer cells. siNC, negative control small interfering RNA.

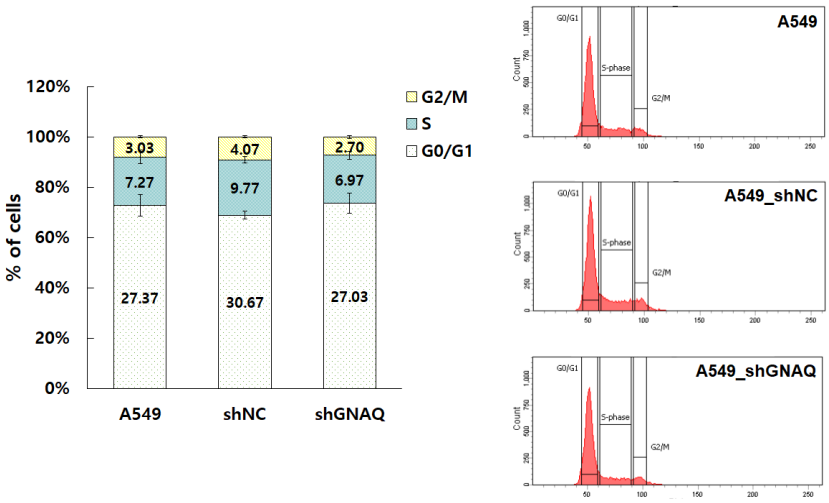

Fig. ba. The effect of G protein subunit alpha Q (GNAQ)-knockdown on the cell cycle and apoptosis. Cells were stained with propidium iodide (PI) for cell cycle analysis and detected by flow cytometry. shNC, negative control shRNA.

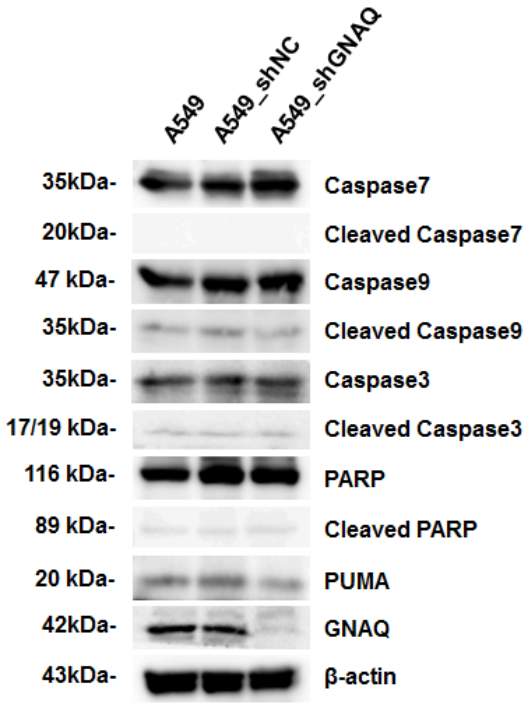

Fig. bb. The effect of G protein subunit alpha Q (GNAQ)-knockdown on the cell cycle and apoptosis. Western blotting for the expression of apoptosis related proteins. PARP, Polyadenosine diphosphate-ribose polymerase; PUMA, p53 upregulated modulator of apoptosis; shNC, negative control shRNA.

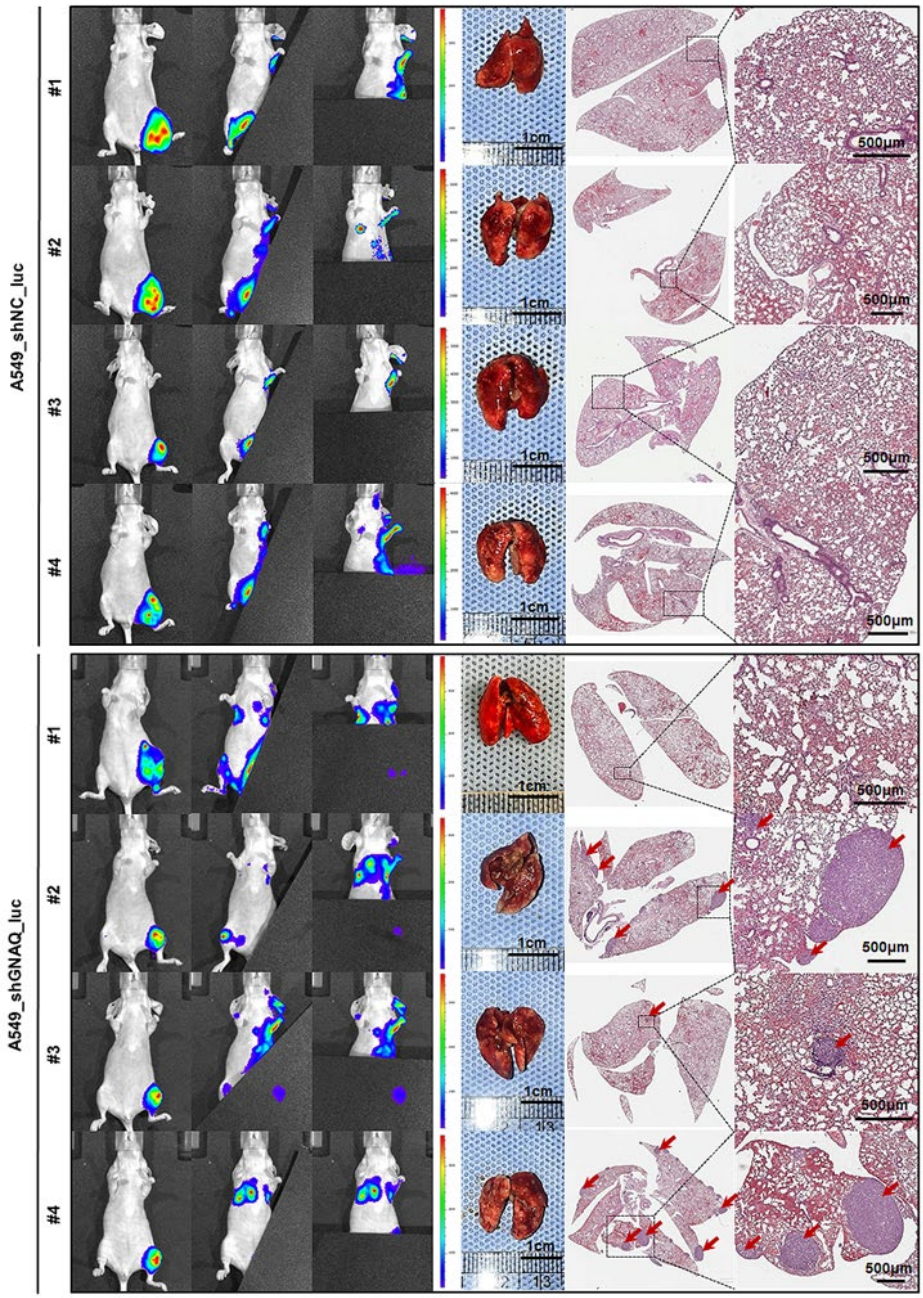

Fig. c. G protein subunit alpha Q (GNAQ)-knockdown promotes in vivo metastasis in mouse model. Anatomical photos of lung tissues from mice injected with A549_shNC_luc and A549_shGNAQ_luc cells. GNAQ-knockdown promotes metastasis to lung and other bone sites in vivo as evidenced by bioluminescent imaging. Lung metastasis was not observed 60 days after injection with

A549_shNC_luc cells. Histological sections of lung were stained by haematoxylin and eosin (H&E). Arrows indicate metastatic nodules in the lungs. Scale bars, 500 μm. shNC, negative control shRNA.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	'Materials and Methods' section(line 86-88, 155-157) 'In vivo assay' section (line 210-219)
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	'Materials and Methods' section (line 85-89, 210-211, 218-220, 226) 'Materials and Methods' section(line 85-89)
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	Not applicable. No exclusions. 'Materials and Methods' section (line 85-89, 210-211, 218-220)
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Randomisation was not used. Confounders were not controlled.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Sung Wook Seo , Ji-yoon Choi
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	'Results'section(line244-245, 256-260, 273-274, 279-281, 298-299, 305, 329-338) Not applicable.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	'Materials and Methods' section (line 132-134, 157-158, 194-197,224-226) 'Materials and Methods' section(line 224-227)
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	'Materials and Methods' section(line 210-212) 'Materials and Methods' section(line 211-212)
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	'Materials and Methods'section(line 87-88, 96-98, 210-215, 218-220) 'Materials and Methods'section (line 86-87, 210-211, 218-219) 'Materials and Methods'section (line 210-211, 218-219) 'Results'section(line232-238, 286-288)
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Not applicable. Not applicable.