Detection of GALNT13 in circulating tumor cells of non-small cell lung cancer (NSCLC) patients. A potential new liquid biopsy biomarker.

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Background

Worldwide, lung cancer is the second cancer with the highest incidence (11.4%) and continues to be the leading cause of death for both sexes (18%), representing approximately one fifth of all cancer deaths[1]. Only 20% of patients are diagnosed at early stage, while most cases present at advanced disease where tissue biopsies are often inaccessible or have not enough material for molecular analysis. Thus, liquid biopsies appear as powerful tools to non-invasively analyze lung cancer phenotype and progression as well as drug resistance[2,3]. Protein O-glycosylation abnormalities are found in most carcinomas. Several clinically useful biomarkers detecting these changes have been developed. The synthesis of mucin-type O-glycans starts in a reaction catalyzed by a complex enzyme family (UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferases; GalNAc-Ts), which has at least 20 members in humans. We previously found that GalNAc-T13 isoenzyme is a strong predictor of poor clinical outcome in neuroblastoma[4] and breast cancer patients[5]. Given that GalNAc-T13 is also expressed in NSCLC[6], this work aimed to evaluate *GALNT13* expression in circulating tumor cells (CTC) as a new potential liquid biopsy biomarker in NSCLC patients.

Methods

Briefly, blood samples were centrifuged to obtain three fractions. The central fraction, containing leucocytes, platelets, and potentially CTCs was separated, and after the remaining erythrocytes were lysed, it was incubated with Trizol® (Sigma Aldrich) for RNA isolation. Subsequently, cDNA was synthesized and *GALNT13* NESTED PCR was conducted in duplicate (Figure 1). The specificity of the method was evaluated by analyzing 20 peripheral blood samples from healthy individuals; while sensitivity was evaluated using a dilution model with cell lines that simulate the clinical sample to be studied (Figure 2). Finally, *GALNT13* expression in CTC was evaluated in 130 NSCLC patients from Hospital Maciel, and CASMU, Montevideo. Results were correlated with clinical-pathologic data and patients' survival.

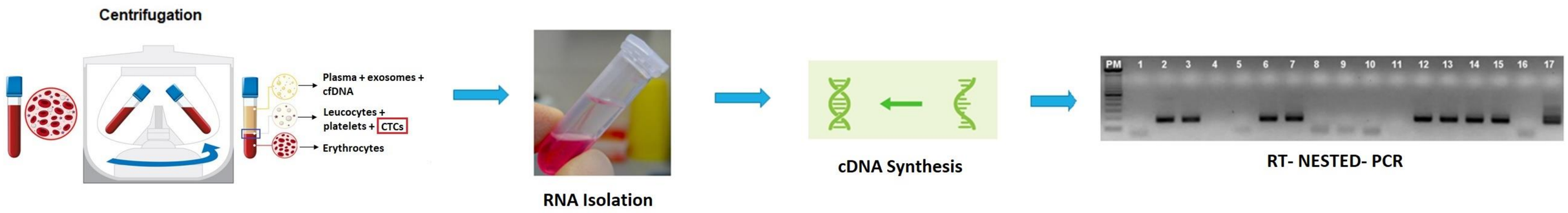
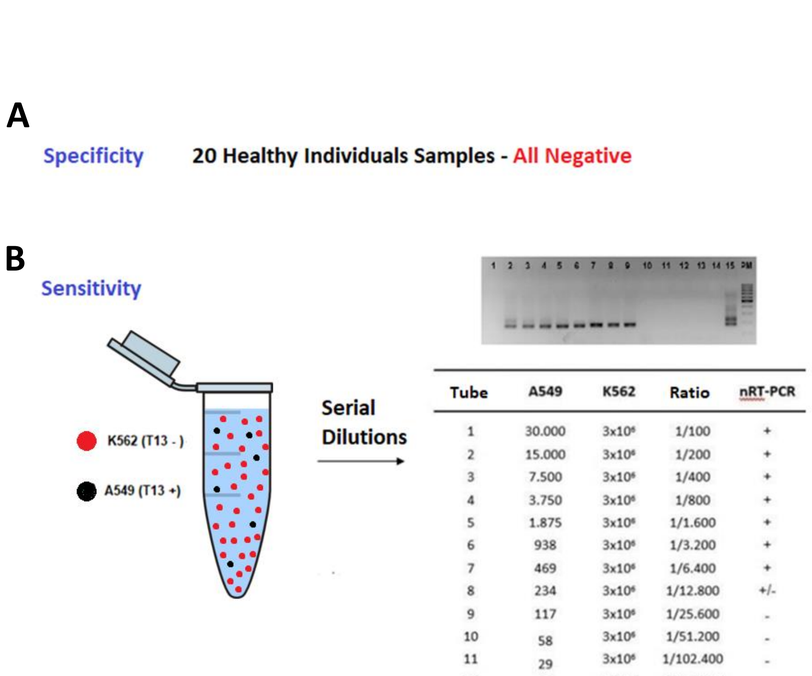


Figure 1: Liquid Biopsy Protocol. Analysis of *GALNT13* expression in peripheral blood of NSCLC patients by RT-NESTED-PCR

Results

No expression of *GALNT13* was found in 20 healthy individuals' samples and the sensitivity of the method was established at 0.01%. *GALNT13* expression was then analyzed in CTCs from 130 NSCLC patients. The median age was 64 years old (ranging 32-84); 66.9% corresponded to males and 33.1% to females. *GALNT13-positive* CTCs were found in 79/130 (60.8%) lung cancer patients. Concerning histological types, *GALNT13* expression was detected in adenocarcinoma patients (42/73; 56.2%) and squamous cell carcinomas patients (21/31; 67.7%). Relating to stages, the enzyme was found in 14/28 (50%) of patients with stages I/II/IIIA and 65/102 (63,7%) of IIIB/IIIC/IV (Table 1). In this preliminary evaluation, we attained three years of follow-up. In early-stage operable patients (I/II/IIIA) *GALNT13* expression was significantly correlated with poor clinical outcomes. In contrast, in NSCLC patients with late stages (IIIB/IIIC/IV) *GALNT13* expression was associated with higher overall survival (Figure 3)



Tube	A549	K562	Ratio	nRT-PCR
1	30.000	3x10⁶	1/100	+
2	15.000	3x10⁶	1/200	+
3	7.500	3x10⁶	1/400	+
4	3.750	3x10⁶	1/800	+
5	1.875	3x10⁶	1/1.600	+
6	938	3x10⁶	1/3.200	+
7	469	3x10⁶	1/6.400	+
8	234	3x10⁶	1/12.800	+/-
9	117	3x10⁶	1/25.600	-
10	58	3x10⁶	1/51.200	-
11	29	3x10⁶	1/102.400	-
12	14	3x10⁶	1/204.800	-

Figure 2: Optimization of a RT-NESTED-PCR method to detect GALNT13 expression. Specificity (**A**) and sensitivity (**B**) evaluation

Table I: *GALNT13* expression in CTCs of NSCLC patients

		GALNT13 (+) n (%)	*GALNT13* (-) n (%)
Patients	n = 130	79 (60.8)	51 (39.2)
Age	Median 64 Range 32-84		
Sex			
	F (n = 43, 35.1%)		
	M (n = 87, 66.9%)		
Histology			
	ADC (n = 73)	42 (56.2)	31 (43.8)
	SCC (n = 31)	21 (67.7)	10 (32.3)
	Others (n = 26)	16 (61.5)	10 (38.5)
Stage			
	I (n = 5)	2 (40)	3 (60)
	II (n = 10)	5 (50)	5 (50)
	IIIA (n = 13)	7 (53.8)	6 (46.2)
	IIIB (n = 12)	9 (75)	3 (25)
	IIIC (n = 9)	6 (66.6)	3 (33.3)
	IV (n = 81)	50 (61.7)	31 (38.3)

CTCs: circulating tumor cells; NSCLC: non-small cell lung cancer; ADC: adenocarcinoma; SCC squamous cell carcinoma

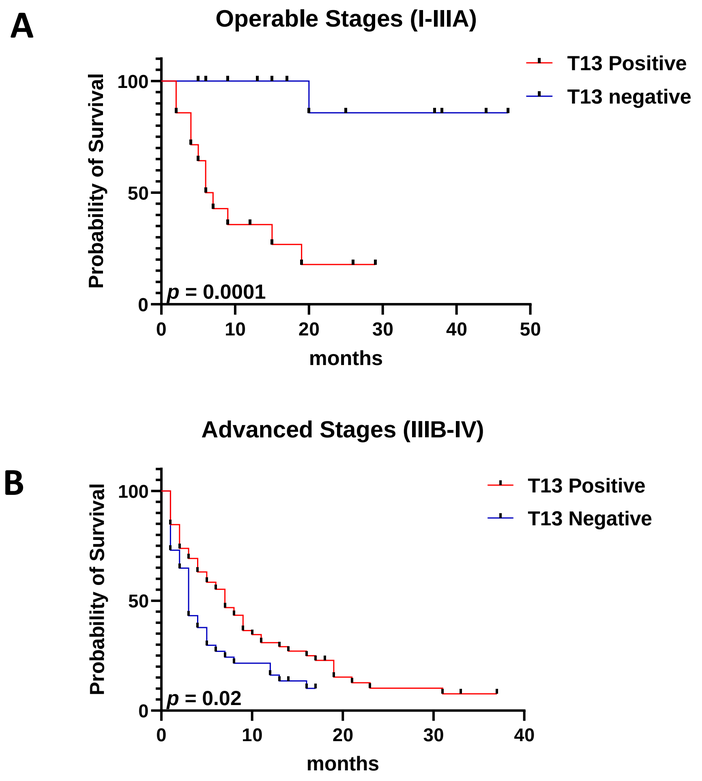


Figure 3: Kaplan–Meier overall survival rates related to *GALNT13* expression. (**A**) Early-stage tumors (stages I, II and IIIA) (n = 28). (**B**) Advanced stage tumors (stages IIIB, IIIC and IV) (n = 102).

Conclusions and Perspectives.

In this preliminary study, we observed that *GALNT13* expression correlated with poor clinical outcomes in lung cancer patients with operable stages (I-IIIA). Unexpectedly, in advanced stages (IIIB-IV), *GALNT13* expression seems to correlate with more favorable outcomes, indicating the need to elucidate the biological role of GalNAc-T13 in NSCLC and to analyze the molecular mechanisms regulating this gene expression. This enzyme could be a novel prognostic biomarker in liquid biopsy for lung cancer patients and a potential candidate for targeted therapy in early stages. Further work is needed to confirm our findings. We are now working on the optimization of a quantitative PCR protocol to evaluate more accurately subtle changes in *GALNT13* expression throughout the disease.

References:
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