GALNT13 detection in liquid biopsy in non-small cell lung cancer (NSCLC) patients.

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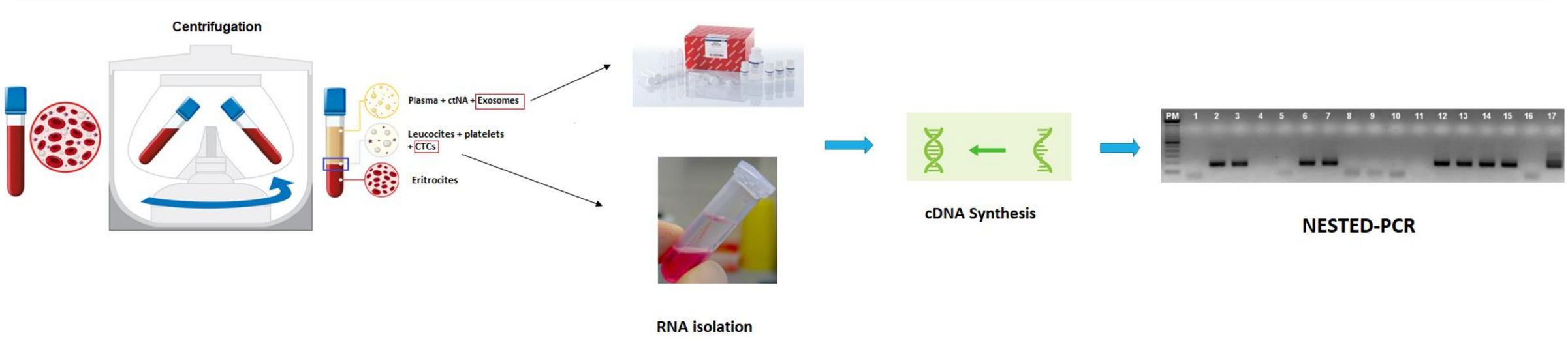
Background

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). Polypeptide-GalNAc-transferases are emerging as novel biomarkers related to cancer and GalNAc-T13 correlates with lung cancer behavior (4,5), In this scenario, the aim of this work was to evaluate *GALNT13* expression (the gene coding for GalNAc-T13) as new potential liquid biopsy biomarker for NSCLC patients.

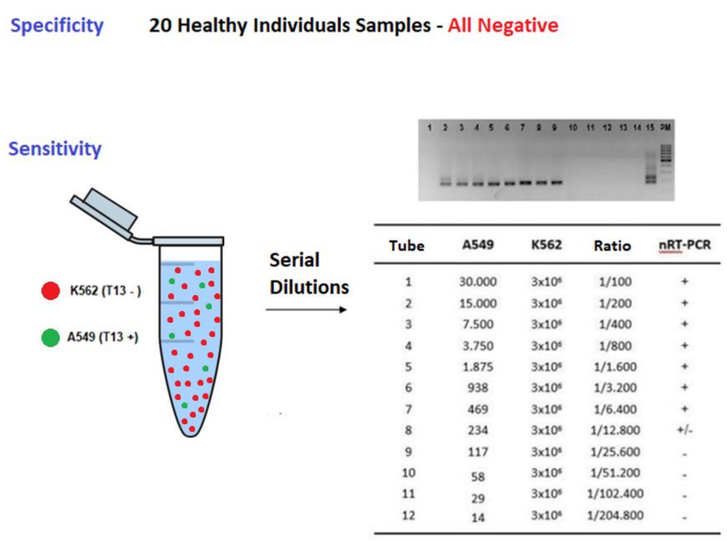
Methods

Blood samples were cetrifuged to obtain three fractions. The central fraction, containing leucocites, platelets and potentialy CTCs was separated, and after remaining erythrocytes were lysed it was incubated with Trizol® (Sigma Aldrich) for RNA isolation. cDNA was syntetized and GALNT13 NESTED PCR was conducted in duplicate. Exosomal RNA was isolated with exoRNeasy Midi kit ® (Qiagen) and a similar protocol was conducted to analyse GALNT13 expression (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).



Results

No expression of *GALNT13* was found in any of the samples from healthy individuals and the sensitivity of nested RT-PCR was established at 0.01% (Figure 2). *GALNT13* expression was then analyzed in CTCs from 112 NSCLC patients. The median age was 64 years old (ranging 32-82), 64.3% corresponded to males and 35.7% to females. *GALNT13*-positive CTCs were found in 68/112 (60.7%) lung cancer patients. Preliminary results show that this glycosyltransferase was detected in patients with different histologic type of NSCLC as well as in different stage of disease. These findings suggest the potential utility for this marker regardless of the stage or histological type (Table 1). In addition, *GALNT13* expression was also analyzed in serial samples from 34 patients (spaced 3-12 months), showing that this enzyme expression could change in the course of the disease. Finally, exosomes from 12 plasma samples were extracted and preliminary results of *GALNT13* expression in plasmatic exosomal RNA was concordant with those obtained for CTCs (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: Sensitivity and Specificity evaluation

Table 1: GALNT13 expression in CTCs of NSCLC patients

		GALNT13 (+) n (%)	*GALNT13* (-) n (%)
Patients	n = 112	68 (60.7)	44 (39.3)
Age	Median 64 Range 32-82		
Sex			
	F (n = 40, 35.7%)		
	M (n = 72, 64.3%)		
Smoking			
	Yes (n = 98)	58 (59.2)	40 (40.8)
	No (n = 9)	5 (55.6)	4 (44.4)
Histology			
	ADC (n = 62)	35 (56.5)	27 (43.5)
	SCC (n = 32)	20 (62.5)	12 (37.5)
	Others (n = 18)	13 (72.2)	5 (27.8)
Stage			
	I / II (n = 18)	13 (72.2)	5 (27.8)
	III A/B (n = 30)	15 (50)	15 (50)
	III C / IV (n = 64)	40 (62.5)	24 (37.5)

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

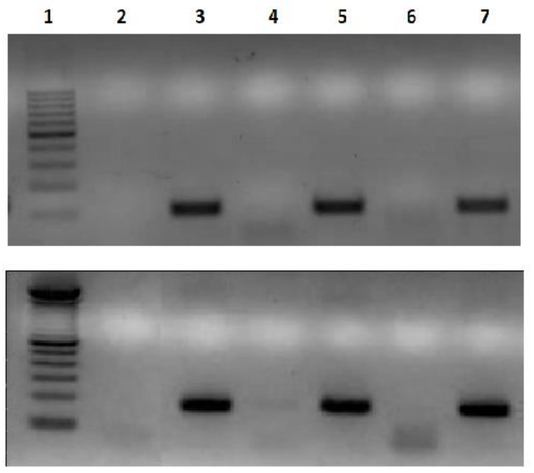


Figure 2: Exosomes vs CTCs. 1- Molar weight; 2- Negative control; 3- LOM 40-S5; 4- LOM 10-S2, 5- LOM-54-S5; 6- LOM 31-S2; 7- Positive control

Conclusions and Perspectives.

In this preliminary study we demonstrate for the first time that *GALNT13* is expressed in CTCs and plasmatic exosomes. *GALNT13*-positive CTCs were found in 68/112 (60.7%) lung cancer patients and it was indifferent for histologic type or stage of the disease, suggesting the potential of this enzyme as a marker across NSCLC patients. In addition, GALNT13 expression was also found in CTCs from serial samples of 34 patients (spaced 3-12 months) and plasmatic exosomes. We are working in optimization of a quantitative PCR protocol in order to evaluate more accurately subtle changes in GALNT13 expression throughout disease. A largest study of GALNT13 expression in exosomes will let us know if this is a better source for GALNT13 evaluation in liquid biopsy.)

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