Helicase-like Transcription Factor (HLTF) expression in Non Small Cell Lung Cancer (NSCLC) is associated with a poor prognosis.

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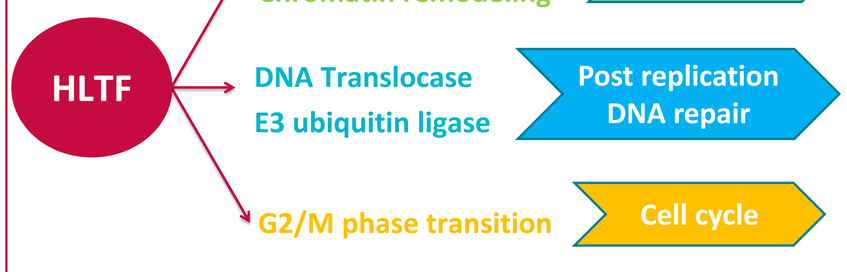
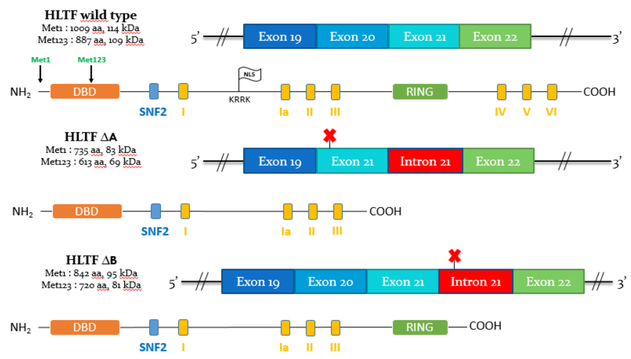
Introduction

Roles of HLTF



HLTF is a Tumor Suppressor Gene

→ *HLTF* promoter hypermethylation in gastric and colon cancers



→ Expression of HLTF truncated forms, resulting from alternative splicing, in head and neck, and cervix cancers (and HeLa cells)

HLTF and lung cancer (NSCLC)

Methylation of the *HLTF* promoter

21/53 (39.6%) patients:
- 7/32 (21.8%) adenocarcinoma
- 9/20 (45.0%) squamous cell carcinoma

→ Shorter survival when HLTF is hypermethylated *vs.* Hypomethylated (p=0.035)

Only ONE study was carried out
No data on HLTF form expression in NSCLC

HLTF is located in 3q25.1-26.1, an area in which genes are frequently amplified in lung squamous cell carcinoma

Objectives

Alterations in *HLTF* expression have shown a clinical relevance in various types of cancer. Our aim is to assess the expression of wild-type (WT) and spliced forms (I21R) of HLTF mRNA in a cohort of 171 patients with resected stage I-II NSCLC.

Methods

In silico analyses

Genomic profiling data (mutation, copy number, DNA methylation, and mRNA expression) were downloaded and analyzed from

The Cancer Genome Atlas — Understanding genomics to improve cancer care
http://cancergenome.nih.gov/

For **HLTF** in **NSCLC** (Lung adenocarcinoma and Lung squamous cell carcinoma)

cBioPortal for Cancer Genomics
http://www.cbioportal.org/public-portal

Additional data were obtained from

COSMIC Catalogue of somatic mutations in cancer
http://cancer.sanger.ac.uk/cosmic

Patient characteristics

171 patients with resected stage I-II NSCLC		
Age	Age<65	57 (33%)
	Age>=65	114 (67%)
Sex	Female	78 (46%)
	Male	93 (54%)
Stage	I	121 (71%)
	II	50 (29%)
Histology	Adenocarcinoma	122 (71%)
	Other:	49 (29%)
	• Squamous cell carcinoma	42
	• Adenosquamous carcinoma	2
	• Large cell carcinoma	4
	• mixed	1

NSCLC Cell lines

Adenocarcinoma : H1693, H2122, H2228, H2279, H1573, H1395, H522, H1792, H838, H1819, H4011, H2291, H2073, H1568, H920, H1993, H4006, HCC827, H3255, H23, H4019, H2126, H1437, H1944, H2009, H2405, H1373, H1355, H1975, HCC2935, A549, H650, H1650 ;

Squamous cell carcinoma: MGH7;

Large cell carcinoma : H661, H4017 ;

Mixed adenosquamous carcinoma : H647 ;

No defined histology : DFC1032, DFC1024, H2036

Non NSCLC lines: HeLa.

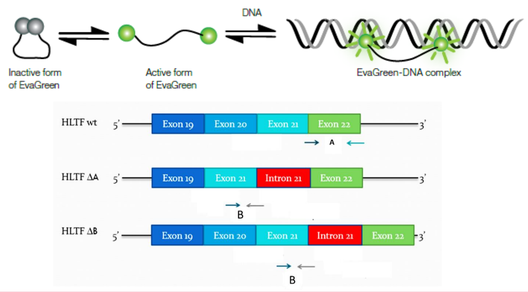
HLTF expression in tumors and cell lines

Reverse transcription – Droplet Digital PCR BIO-RAD

I RT 150 ng RNA → cDNA II ddPCR 22.5 ng cDNA X 3

1. Droplet generation 2. PCR 3. Droplet reading

→ Quantitative evaluation of HLTF mRNAs
→ High sensitivity



Statistics

Measurement reliability
- Intraclass correlation coefficient (ICC)

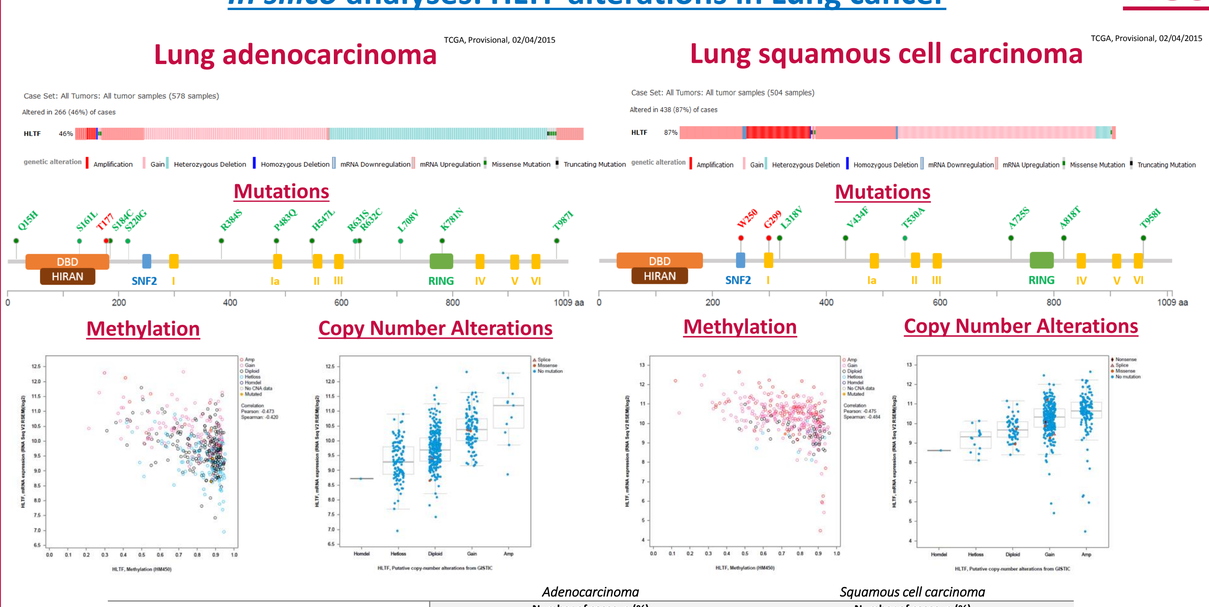
Outcome analysis
- Cox proportional hazard regression
- HTLF form expressions dichotomized at their median: log rank test
- Kaplan-Meier method for survival and disease-free survival (DFS)

P Values: Wald test

Association of HLTF and clinical factors
- Mann-Whitney test

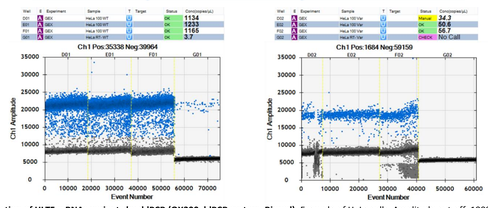
Results

In silico analyses: HLTF alterations in Lung cancer



		Adenocarcinoma	Squamous cell carcinoma
		Number of cases, n (%)	Number of cases, n (%)
	cBioportal	578	504
	Mutation	13 (2.0%)	8 (1.0%)
Copy number alterations (CNA)			
	Homozygous deletion	1 (0.1%)	1 (0.2%)
	Heterozygosity loss	113 (19.8%)	14 (2.8%)
	Diploid	255 (44.8%)	67 (13.5%)
	Gain	113 (19.8%)	281 (56.9%)
	High level of amplification	11 (1.9%)	127 (25.7%)
mRNA expression level			
	High expression	43 (7.5% ; 6 with CNV alteration)	141 (28.6% ; 140 with CNV alteration and 1 with mutation)
	Low expression	0 (0.0%)	6 (1.2% ; 4 with CNV alteration)

HLTF expression by RT-ddPCR



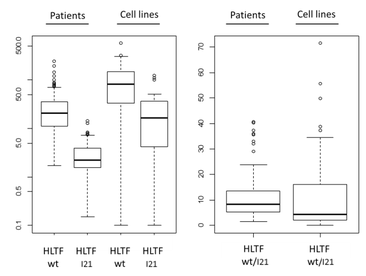
Detection of HLTF mRNAs variants by ddPCR (QX200 ddPCR system, Biorad). Example of HeLa cells. Amplitude cut-off: 10000 units.

Result reliability

	ICC 1 replicate/sample	ICC 3 replicates/sample
Cell lines HLTF WT	0.732	**0.891**
Cell lines HLTF I21R	0.829	**0.936**
Patients HLTF WT	0.955	**0.984**
Patients HLTF I21R	0.647	**0.846**

ICC > 0.8 → results are reliable

Distribution of *HLTF* expression



Association of *HLTF* expression with outcome

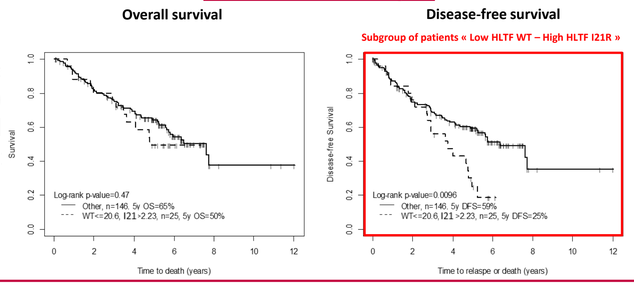
Univariate analysis

Outcome			Dichotomized		Continuous, original scale		Continuous, log scale	
	Category	n	Estimate at 5 years	Logrank p-value	HR (95% CI)*	Wald p-value	HR (95% CI)	Wald p-value
Survival	WT<=20.6	86	61%	0.63	1.03 (0.97-1.1)	0.34	1.13 (0.87-1.46)	0.36
	WT>20.6	85	64%					
	I21R <=2.23	83	63%	0.65	1.03 (0.92-1.15)	0.61	1.08 (0.76-1.54)	0.66
	I21R >2.23	88	61%					
	WT/I21R <=8.25	86	63%	0.6	1.02 (0.99-1.05)	0.27	1.17 (0.81-1.68)	0.41
	WT/I21R >8.25	85	61%					
DFS	WT<=20.6	86	48%	0.14	1.01 (0.95-1.08)	0.72	1.04 (0.82-1.33)	0.73
	WT>20.6	85	61%					
	I21R <=2.23	83	58%	0.73	1.02 (0.93-1.13)	0.64	1.16 (0.84-1.6)	0.38
	I21R >2.23	88	49%					
	WT/I21R <=8.25	86	51%	0.47	1 (0.96-1.03)	0.93	0.92 (0.65-1.3)	0.64
	WT/I21R >8.25	85	57%					

Association of HLTF with survival and disease-free survival (DFS). **Note: HRs for HLTF WT represent the increase of the hazard for 10 units increase in the HLTF WT.*

→ No significant association of any variable with survival and DFS.

Multivariate analysis



Association of *HLTF* expression with clinical factors

HLTF WT and HLTF I21R expressions and their relative expression (ratio WT/I21R and groups of co-expression) were tested for their association with patient clinical factors (age, sex, histology and stage). No significant association was shown.

Conclusion

By *in silico* database analysis, HLTF alterations were found more frequently in lung squamous cell carcinoma than in lung adenocarcinoma. Those alterations include mutations, copy number alterations, methylation and mRNA expression modifications. Using cell lines and patient samples, expression of both HLTF WT and variant (I21R) forms were detected in NSCLC. The combination of a low HLTF WT expression with a high HLTF variant I21R expression is associated with a poor DFS both in univariate and multivariate analyses. These findings endorse our hypothesis that HLTF variant I21R expression could be a predictor of poor outcome for patients.

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