TERT PROMOTER MUTATIONS CORRELATE WITH A MORE ADVANCED STAGE AT DIAGNOSIS AND WITH A POORER PROGNOSIS IN DIFFERENTIATED THYROID CANCER

C. Colombo, M. Muzza, S. Rossi, V. Cirello, M. Perrino, D. Tosi, B. Vigo, S. De Leo, G. Bulfamante, E. Magnani, E. Pignatti, M. Simoni, L. Vicentini, L. Fugazzola

Dept. of Clinical Sciences and Community Health, University of Milan, Endocrine Unit, Fondazione IRCCS Ca' Granda, Milan; Dept. of Biomedicine, Metabolism and Neural Sciences, University of Modena and Reggio Emilia

TELOMERASE

Telomerase is a ribonucleoprotein polymerase that maintains telomere ends and plays a role in cellular senescence, being repressed in postnatal somatic cells. Mutations **C228T** and **C250T** in promoter of telomerase reverse transcriptase (TERT) were recently reported in human cancers.

TERT PROMOTER MUTATIONS IN THYROID CANCERS

PAPILLARY (PTC): 138/1297 $TERT^{228/250}$ **(10.6%)**

FOLLICULAR (FTC): 48/271 $TERT^{228/250}$ **(17.7%)**

POORLY DIFFERENTIATED/ ANAPLASTIC (PDTC/ATC): 104/257 $TERT^{228/250}$ **(40.4%)**

MEDULLARY (MTC): 0/110 $TERT^{228/250}$ **(0%)**

FAMILIAL PTC: 0/18 $TERT^{228/250}$ **(0%)**

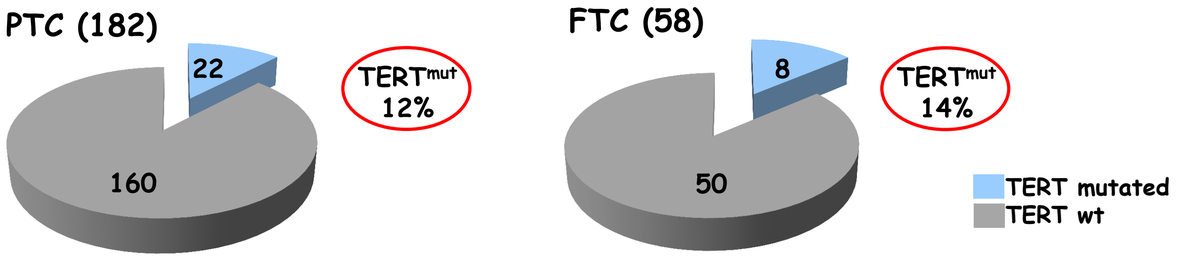
THYROID NODULAR BENIGN DISEASES: 0/291 $TERT^{228/250}$ **(0%)**

Landa et al,2013; Vinagre et al, 2013; Liu T et al, 2013; Liu X et al, 2013; Melo et al, 2013; Liu X et al, 2014

AIM OF THE STUDY: A) To explore TERT promoter mutations in a large series of PTCs and FTCs and to correlate them with clinical and prognostic data; B) To investigate TERT expression and localization in neoplastic and normal thyroid tissues

RESULTS

PREVALENCE OF TERT PROMOTER MUTATIONS IN DIFFERENT THYROID TUMORS



CLINICAL FEATURES OF THE 240 TERT MUTATED AND WILD-TYPE (WT) DTC

PTC + FTC		TERT mutated (n=30) (12.5%)	TERT WT (n=210) (87.5%)	P
Age at diagnosis (years)	mean	59.6	47.3	**0.002**
	range	29-82	14-85	
Female gender		20/30 (66.6%)	151/210 (71.9%)	0.7
Mean tumor size (mm)		31	25	0.11
Tumor (T)	T1	7/30 (23.3)	78/210 (37.1)	0.49
	T2	5/30 (16.6%)	24/210 (11.4%)	
	T3	16/30 (53.3%)	101/210 (48%)	
	T4a	2/30 (6.6%)	7/210 (3.3%)	
Multifocality		12/30 (40%)	86/210 (40.9%)	0.9
Extrathyroid invasion		16/30 (53.3%)	109/210 (51.9%)	0.9
Lymph-nodes (N1)		12/30 (40%)	87/210 (41.4%)	0.96
Stage	I	10/30 (33.3%)	126/210 (60%)	0.068
	II	4/30 (13.3%)	12/210 (5.7%)	
	III	9/30 (30%)	49/210 (23.3%)	
	IV	7/30 (23.3%)	23/210 (10.9%)	
Outcome	persistence or recurrence	13/30 (43.3%)	40/210 (19%)	**0.0057**

In PTCs and FTCs, considered separately or pooled (PTCs + FTCs), TERT mutations were found to be significantly associated with an **OLDER AGE AT DIAGNOSIS** and a **WORST OUTCOME**

CORRELATION OF TERT AND BRAF STATUS IN PRIMARY TUMORS

	$TERT^{MUT}$	$TERT^{WT}$	
$BRAF^{V600E}$ (TOT 64)	10	54	→ 10/64 **(15.6%)** BRAF+ andTERT+
$BRAF^{WT}$ (TOT 118)	12	106	→ 12/118 **(10.2%)** $BRAF^{WT}$ and TERT+

P=0.4

Although the difference was not significant, TERT mutations were more frequent in $BRAFV^{600E}$ than in $BRAF^{WT}$ cases

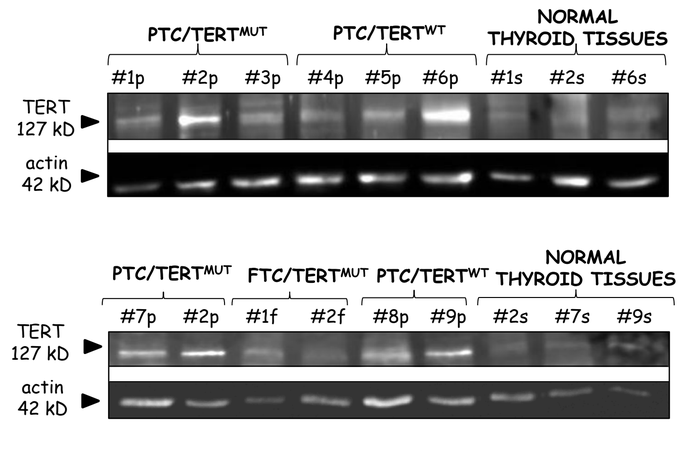
TERT AND BRAF MUTATIONS IN LYMPH-NODAL METASTASES

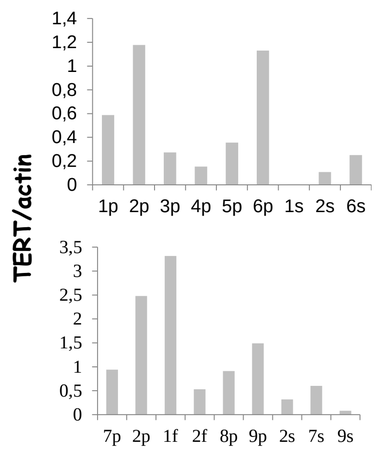
#	PRIMARY THYROID TUMOUR		LYMPH NODE METASTASES	
	BRAF V600E	*TERT* C228T/C250T	*BRAF* V600E	*TERT* C228T/C250T
1,2,3	+	-	+	-
4	-	-	-	+ C228T
5	+	-	-	-
6,7	-	-	-	-
8	-	+ C228T	+	+ C228T

- the molecular pattern was IDENTICAL among the primary tumor and the lymph-node metastases in 5 cases
- The molecular pattern was **DISCREPANT** in 3 patients

This finding could be due to:
- ✓ the **selection of mutant alleles** during tumor progression
- ✓ or to the heterogeneous pattern of tumoral cells in the primary tumor with only **some subclones** able to metastatize

WB ANALYSIS OF TERT MUTATED AND TERT WT DIFFERENTIATED THYROID CANCERS AND OF NORMAL CONTROL THYROID TISSUES



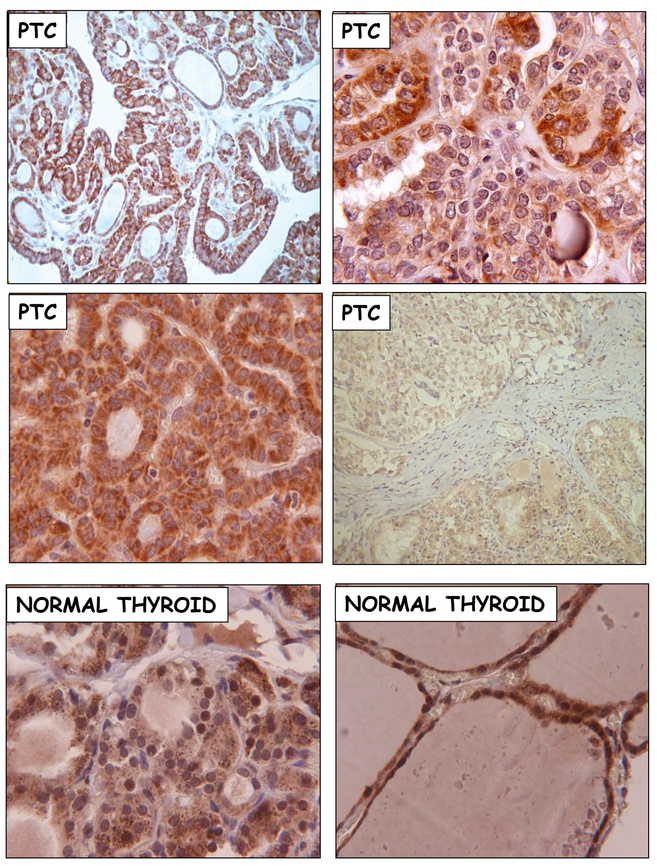


Mean TERT/actin **in tumors**: **1.11**
Mean TERT/actin **in normal tissues**: **0.23**
P=0.008

Mean TERT/actin **$TERT^{MUT}$** tumors: **1.33**
Mean TERT/actin in **$TERT^{WT}$** tumors: : **0.81**
P=0.3

- Mean TERT/actin expression in TUMORS was significantly **higher** than in NORMAL TISSUES
- Moreover, **$TERT^{MUT}$ tumors had a higher expression of the protein**, though not at a significant level, with respect to $TERT^{WT}$ cases

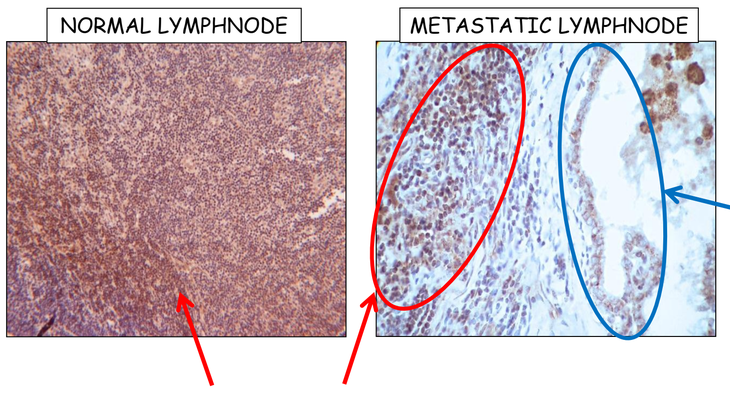
IMMUNOHISTOCHEMISTRY ANALYSES OF TERT EXPRESSION IN $TERT^{MUTATED/WT}$ DTC AND NORMAL CONTROL THYROID TISSUES



TUMOR TISSUES
↓
CYTOPLASMIC STAINING:
- diffuse
- in aggregates
- in dots

NORMAL TISSUES
↓
BOTH
CYTOPLASMIC AND NUCLEAR POSITIVITY

IMMUNOHISTOCHEMISTRY ANALYSES OF TERT EXPRESSION IN NORMAL AND METASTATIC LYMPHNODES



CONCLUSIONS

- **The PROGNOSTIC VALUE of telomerase mutations was shown in a large series of differentiated thyroid tumors**
- **Telomerase mutations were demonstrated to promote a HIGHER EXPRESSION and the EXCLUSION FROM THE NUCLEUS of the protein in neoplastic tissues, that might contribute to cancer progression through a mechanism independent from telomeres elongation**