ESMO TAT
VIRTUAL CONGRESS
Targeted Anticancer Therapies

Emerging role of Telomeric repeat-containing RNA TERRA in hepatocellular carcinoma

M. Manganelli[1], I. Grossi[1], J. Corsi[2], K. Jurikovà[2], E. Cusanelli[2], V. D'Agostino[2], S. Molfino[3], G. Baiocchi[3], N.Portolani[3], A. Salvi[1], G. De Petro[1]

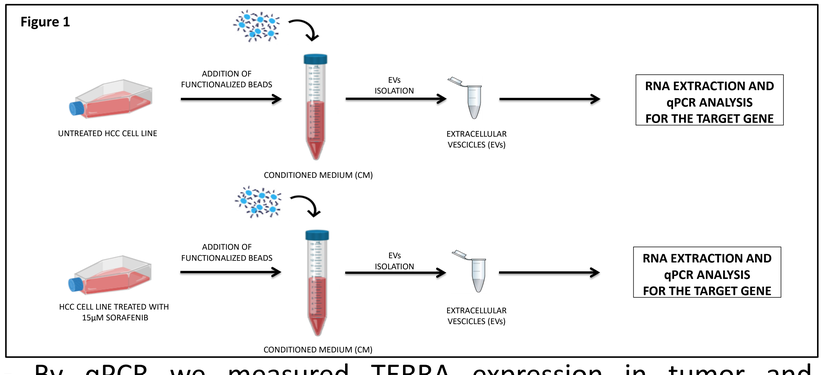
[1]Department of Molecular and Translational Medicine, Division of Biology and Genetics, University of Brescia, Brescia, Italy. m.manganelli@unibs.it
[2]Department of Cellular, Computational and Integrative Biology, CIBIO, University of Trento, Trento, Italy.
[3]Department of Clinical and Experimental Sciences, Surgical Clinic, University of Brescia, Brescia, Italy.

BACKGROUND

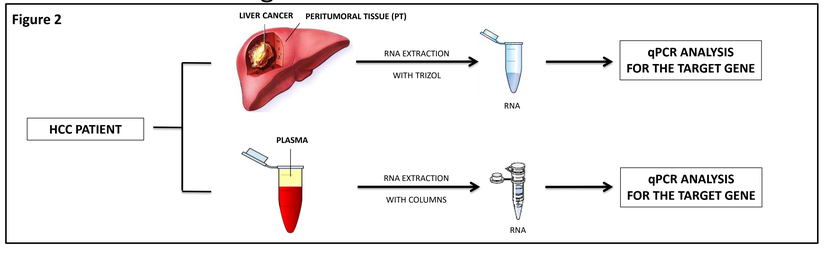
- Hepatocellular carcinoma (HCC) is the most frequent primary tumor of the liver and the 3rd cause of cancer-related deaths[1].
- The identification of candidate molecular targets and biomarkers in HCC clinical practice are needed.
- The signatures of aberrant long non-coding RNAs (lncRNAs) expression in HCC tissues, their extracellular release and stability had led to their exploration as diagnostic and prognostic tools as well as potential therapeutic targets[2].
- Telomeric-Repeat Containing RNA (TERRA) consists of 100nt-9Kb subtelomeric-derived transcripts able to base-pair with TERC RNA, acting as telomerase allosteric inhibitor[3,4]. Little is known on the role of lncRNA TERRA in HCC.

METHODS

- By nickel-based isolation method (NBI) we isolated from the conditioned medium (CM) of HA22T/VGH cells the extracellular vesicles (EVs), subsequently analyzed by qNANO instrument[5].



- By qPCR we measured TERRA expression in tumor and peritumoral (PT) tissues of HCC patients (N=25), as well as in plasma and in HCC cells. HCC patients did not receive any treatment before surgical resection.



RESULTS

- Global TERRA expression was significantly downregulated in HCC vs PT tissues (p=0.025) and ROC analysis revealed a significant ability to distinguish HCC from PT (p=0.03).

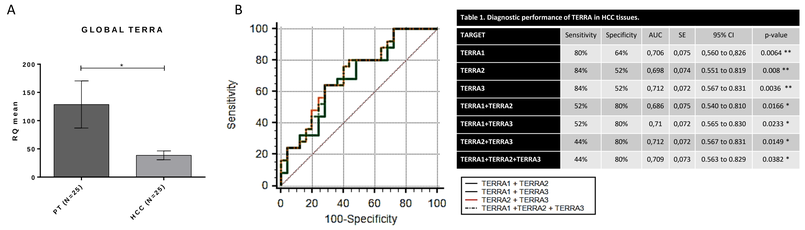


Table 1. Diagnostic performance of TERRA in HCC tissues.

TARGET	Sensitivity	Specificity	AUC	SE	95% CI	p-value
TERRA1	80%	64%	0,706	0,075	0,560 to 0,826	0.0064 **
TERRA2	84%	52%	0,698	0,074	0.551 to 0.819	0.008 **
TERRA3	84%	52%	0,712	0,072	0.567 to 0.831	0.0036 **
TERRA1+TERRA2	52%	80%	0,686	0,075	0.540 to 0.810	0.0166 *
TERRA1+TERRA3	52%	80%	0,71	0,072	0.565 to 0.830	0.0233 *
TERRA2+TERRA3	44%	80%	0,712	0,072	0.567 to 0.831	0.0149 *
TERRA1+TERRA2+TERRA3	44%	80%	0,709	0,073	0.563 to 0.829	0.0382 *

Figure 3. Expression of TERRA in solid biopsies. A) TERRA relative quantification mean comparison between PT and HCC. Wilcoxon paired two-tailed t-test. B) ROC curve analysis of TERRA. Sensitivity: percent of correctly classified HCC patients. Specificity: percent of correctly classified non-HCC. AUC: Area Under the ROC Curve. CI: Confidence Interval. *p<0.05, **p<0.01

- Extracellular TERRA transcripts were significantly higher in plasma of HCC patients compared with healthy subjects and logistic regression model strongly evidenced the potential diagnostic ability of circulating TERRA (AUC=0.76; 95% CI= 0.624-0.873; p=0.0004).

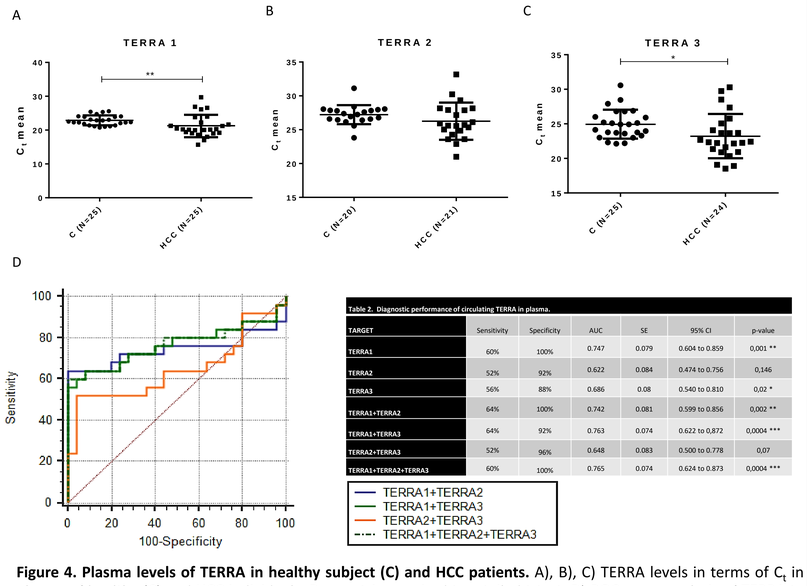


Table 2. Diagnostic performance of circulating TERRA in plasma.

TARGET	Sensitivity	Specificity	AUC	SE	95% CI	p-value
TERRA1	60%	100%	0.747	0.079	0.604 to 0.859	0,001 **
TERRA2	52%	92%	0.622	0.084	0.474 to 0.756	0,146
TERRA3	56%	88%	0.686	0.08	0.540 to 0.810	0,02 *
TERRA1+TERRA2	64%	100%	0.742	0.081	0.599 to 0.856	0,002 **
TERRA1+TERRA3	64%	92%	0.763	0.074	0.622 to 0,872	0,0004 ***
TERRA2+TERRA3	52%	96%	0.648	0.083	0.500 to 0.778	0,07
TERRA1+TERRA2+TERRA3	60%	100%	0.765	0.074	0.624 to 0.873	0,0004 ***

Figure 4. Plasma levels of TERRA in healthy subject (C) and HCC patients. A), B), C) TERRA levels in terms of C_t in plasma of healthy (C) and HCC individuals. Parametric unpaired two-tailed t-test. D) ROC curve analysis of TERRA to discriminate HCC from healthy individuals. Sensitivity: percent of correctly classified HCC patients. Specificity: percent of correctly classified non-HCC. AUC: Area Under the ROC Curve. CI: Confidence Interval. *p<0.05, **p<0.01, ***p<0.001.

- HA22T/VGH cells expressed TERRA, but most of the transcripts are released into the CM, also encapsulated in EVs. Treatment of HCC cells with the multi-kinase inhibitor (KI) sorafenib significantly increased TERRA expression (p=0.001) and decreased (p=0.01) its release in EVs.

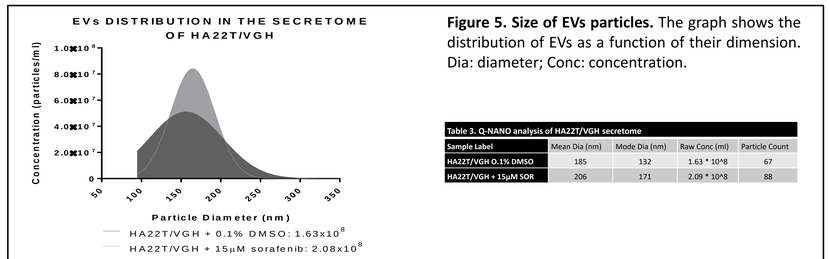


Figure 5. Size of EVs particles. The graph shows the distribution of EVs as a function of their dimension. Dia: diameter; Conc: concentration.

Table 3. Q-NANO analysis of HA22T/VGH secretome

Sample Label	Mean Dia (nm)	Mode Dia (nm)	Raw Conc (ml)	Particle Count
HA22T/VGH 0.1% DMSO	185	132	1.63 * 10^8	67
HA22T/VGH + 15μM SOR	206	171	2.09 * 10^8	88

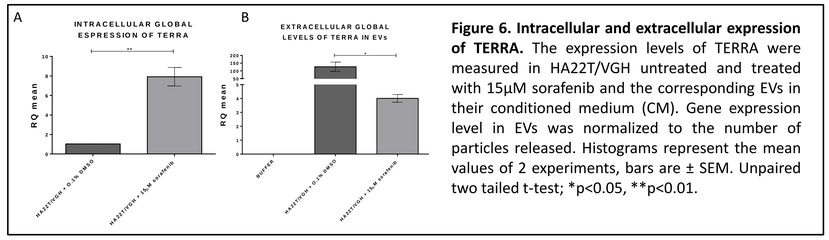


Figure 6. Intracellular and extracellular expression of TERRA. The expression levels of TERRA were measured in HA22T/VGH untreated and treated with 15μM sorafenib and the corresponding EVs in their conditioned medium (CM). Gene expression level in EVs was normalized to the number of particles released. Histograms represent the mean values of 2 experiments, bars are ± SEM. Unpaired two tailed t-test; *p<0.05, **p<0.01.

CONCLUSION

- Our results provide evidence on TERRA dysregulation in tissues and liquid biopsy of HCC patients, focusing on a novel potential non-invasive biomarker of diagnosis and downstream target of the KI.
- TERRA detected in the EVs of HCC cells open a new field of cancer research to comprehend its role at the extracellular level.

REFERENCES

[1]Bray F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
[2]EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018;69(1):182-236.
[3]Azzalin C.M. et al. Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science. 2007;318(5851):798-801.
[4]Schoeftner S. et al. Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat Cell Biol. 2008;10(2):228-236.
[5]Notarangelo M. et al. Ultrasensitive detection of cancer biomarkers by nickel-based isolation of polydisperse extracellular vesicles from blood. *EBioMedicine.* 2019;43:114-126.

10P