The cellular and extracellular forms of the non-coding RNAs *TERRA* and *TERC* and *TERT* mRNA are dysregulated in human hepatocellular carcinoma

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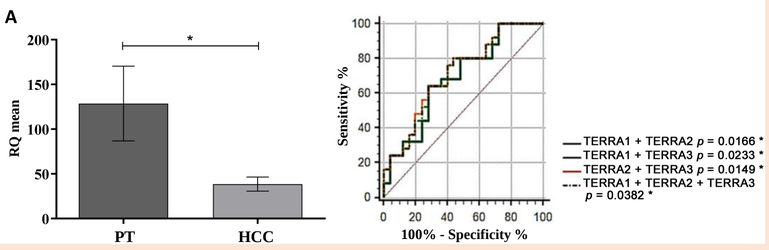
Background **Telomeric repeat-containing RNA** -***TERRA***- consists of different subtelomeric-derived transcripts (from 100 to 10 Kb in length) containing the canonical telomeric repeat sequence UUAGGG and sequences unique to the subtelomeric region of each chromosome. *TERRA* interacts with the telomerase core components (telomerase RNA component -*TERC*- and telomerase reverse transcriptase -TERT) and it is considered a **regulator of telomere homeostasis** by blocking the telomerase activity and altering the telomere length. Growing evidence indicates that *TERRA* is implicated in tumorigenesis, but little is known about its role in **human hepatocellular carcinoma** (HCC). Here, we determined the expression levels of *TERRA*, *TERC* and *TERT* mRNA in HCC solid biopsies as well as in the plasma and we explored their cellular and extracellular levels in HCC cell lines, sensitive and resistant to the anticancer drug sorafenib (a multi-kinase inhibitor).

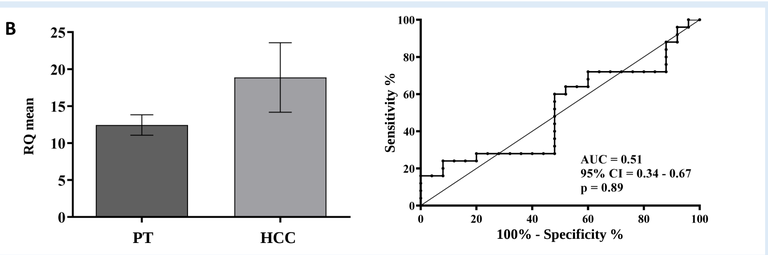
Materials and Methods HCC tissues as well as the corresponding peritumoral (PT) tissues were obtained from 25 HCC patients. Plasma was isolated from blood of healthy individuals (n=25) and of HCC patients (n=25). *TERRA* levels were obtained by qPCR as a mean of relative quantifications of *TERRA* from different telomeres (TERRA 1_2_10_13q: **TERRA1**, 15q: **TERRA2**, XpYp: **TERRA3**). *TERC* and *TERT* mRNA levels were measured by **qPCR** in solid biopsies and in cells and by **ddPCR** in plasma. Extracellular vesicles (EVs) were isolated from the secretome of HCC cells using the nickel-based isolation method (PMID: 33654737) and subsequently analyzed by Q-NANO instrument (IZON).

TERRA, *TERC* and *TERT* mRNA are dysregulated in HCC tissues

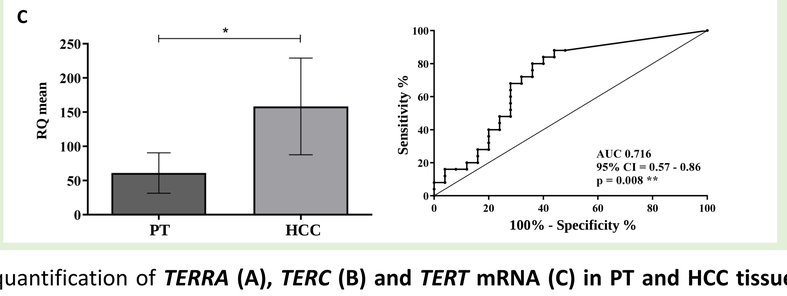
Tissue expression of *TERRA*



Tissue expression of *TERC*



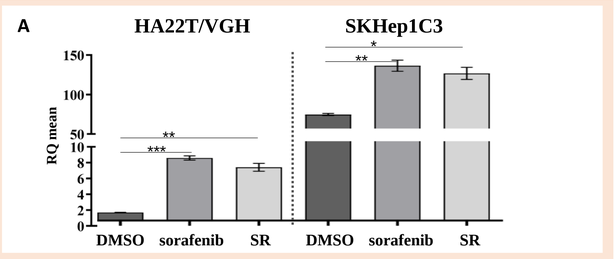
Tissue expression of *TERT* mRNA



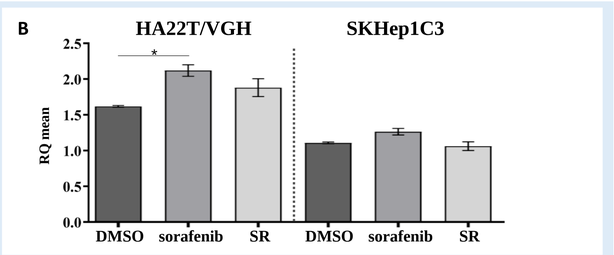
Histograms represent the relative quantification of ***TERRA* (A), *TERC* (B) and *TERT* mRNA (C) in PT and HCC tissues**. Wilcoxon test was used; *p<0.05. On the right, ROC curve analysis reveals for each transcript the capability to discriminate HCC from PT tissues. For *TERRA*, the logistic regression model was applied to evaluate the diagnostic performance of individuals (*TERRA1*, *TERRA2*, *TERRA3*) as well as combinations of classifiers. *p<0.05, **p<0.01.

Sorafenib increases cellular levels of *TERRA, TERC* and *TERT* mRNA in HCC cells

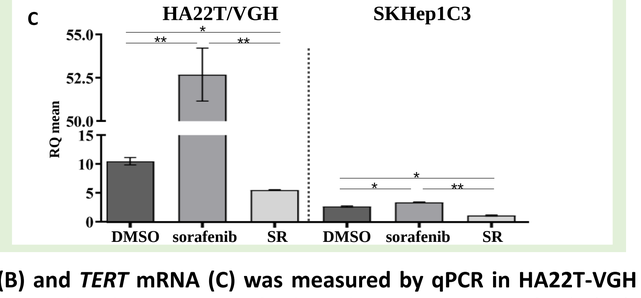
Intra-cellular expression of *TERRA*



Intra-cellular expression of *TERC*



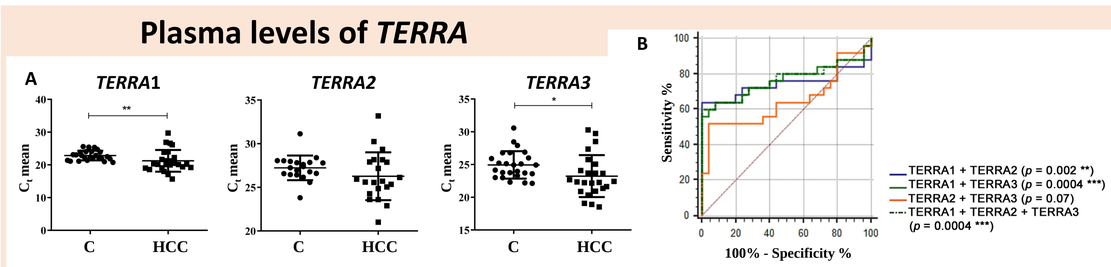
Intra-cellular expression of *TERT mRNA*



The expression level of ***TERRA* (A), *TERC* (B) and *TERT* mRNA (C) was measured by qPCR in HA22T-VGH and SKHep1C3 cells** untreated (DMSO), treated with 15 µM sorafenib and resistant to sorafenib (SR). Unpaired t-test was used; *p<0.05, **p<0.01, ***p<0.001.

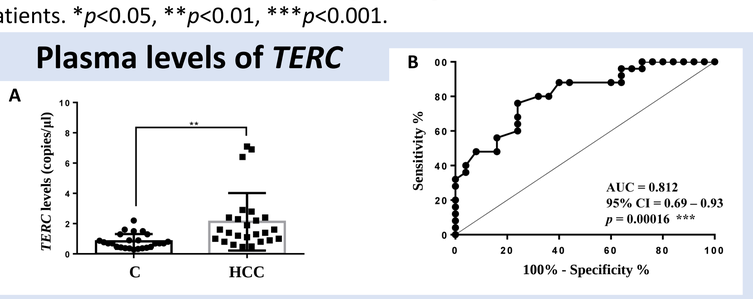
TERRA and *TERC* are up-regulated in plasma from HCC patients

Plasma levels of *TERRA*



***TERRA* levels in terms of C_t in plasma of healthy and HCC individuals.** (**A**) *TERRA* expression from different telomers was analyzed including *TERRA*1, TERRA2, and TERRA3. C, healthy subjects; HCC, HCC patients. Unpaired t-test was used. (**B**) ROC curves constructed with the logistic regression model for discriminating between healthy individuals and HCC patients. *p<0.05, **p<0.01, ***p<0.001.

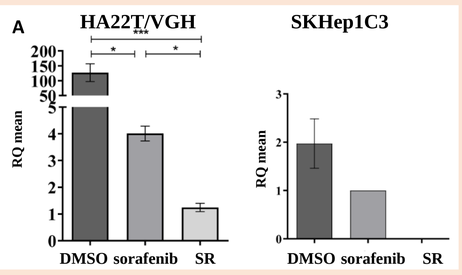
Plasma levels of *TERC*



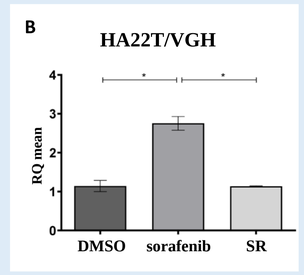
***TERC* levels in terms of copies/µL in plasma of control subjects and HCC patients (A).** C, healthy subjects; HCC, HCC patients. Unpaired t-test was used. ROC curve analysis of *TERC* to discriminate HCC from healthy individuals **(B)**. **p<0.01; ***p<0.001. *TERT mRNA* was undetectable in plasma samples.

TERRA and TERC are secreted in EVs by HCC cells

Extracellular levels of *TERRA*



Extracellular levels of *TERC*



***TERRA* (A) and *TERC* (B) level measured by qPCR** and normalized to the n. of particles in **EVs** from HA22T-VGH and SKHep1C3 cells untreated (DMSO), treated with 15 µM sorafenib and resistant to sorafenib (SR). Unpaired t-test was used; *p<0.05, ***p<0.001. *TERC* was undetectable in EVs from SKHep1C3 cells; EVs from both cell lines did not display *TERT* mRNA.

Conclusions **Our results provide novel insights on the contribution of these transcripts as innovative non-invasive molecular indicators of HCC and the involvement of *TERRA* and *TERC* in EVs of HCC cells in response to sorafenib treatment and in the development of the resistance (PMID:35682861).**

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