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FTIR SPECTROSCOPY FOR CANCER-DERIVED EXTRACELLULAR VESICLES DISCRIMINATION: TOWARDS LIQUID BIOPSY TOOL FOR CANCER DIAGNOSIS

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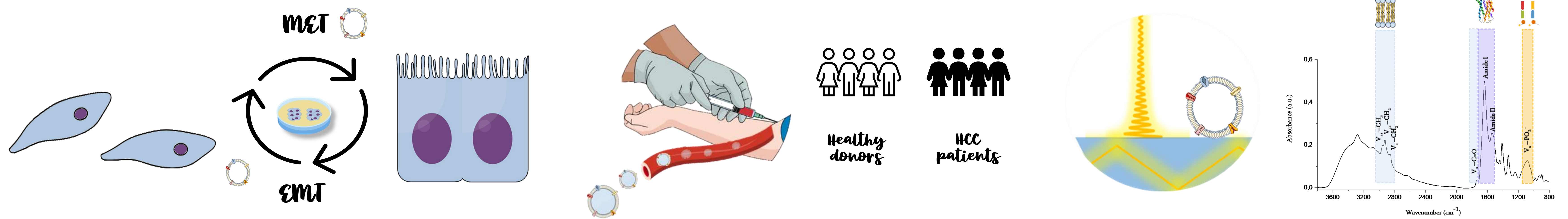
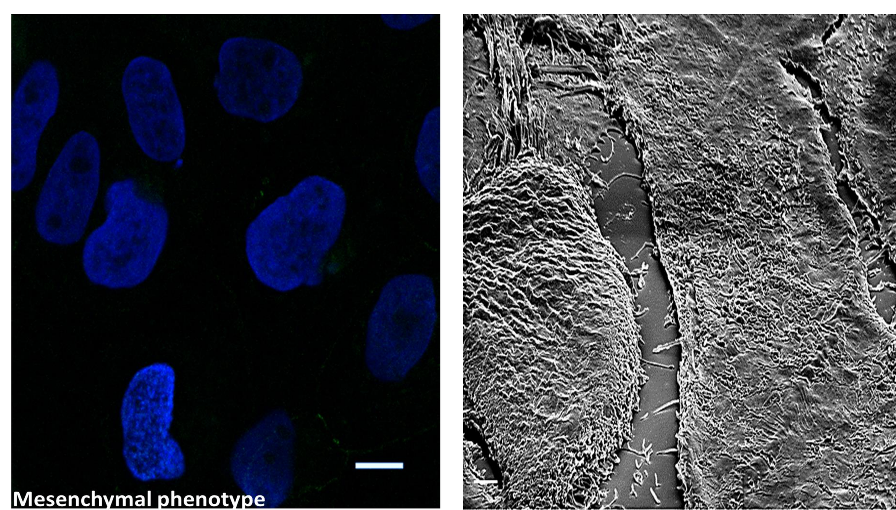
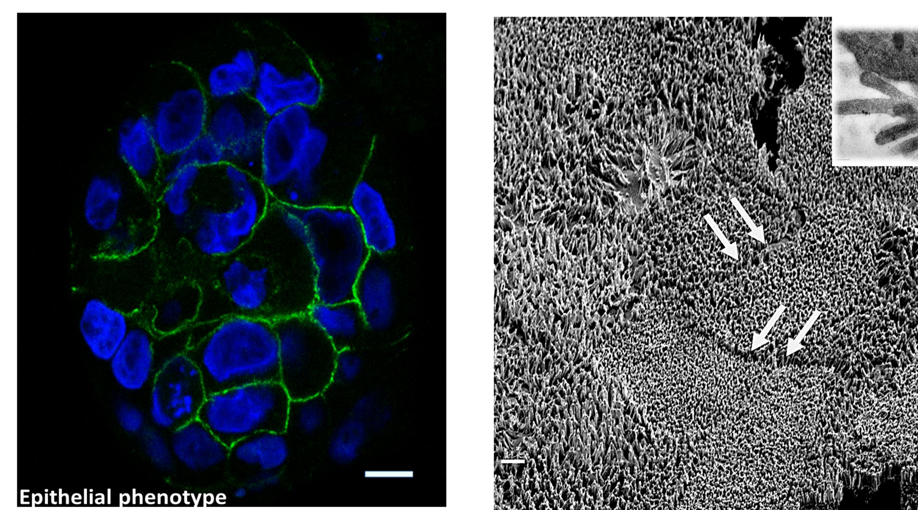


Extracellular Vesicles (EVs) are recently getting much attention in personalized medicine, with a focus on cancer. In this scenario, the development of effective methods for a label-free characterization of these molecules is highly demanded. In this work, we used FTIR[1] in the mid-IR range to investigate the composition of EVs and discriminate their origin. First, the efficacy of the technique was tested on an in vitro model using the human colorectal adenocarcinoma intestinal cell line Caco-2 to discriminate EVs from two different cell states through an induced epithelial-mesenchymal reverse transition (MET)[2]. Secondly, FTIR spectroscopy was applied to discriminate EVs in the serum from patients diagnosed with hepatocellular carcinoma (HCC) of metabolic origin and healthy donors[3].

Characteristics of in-vitro and clinical study





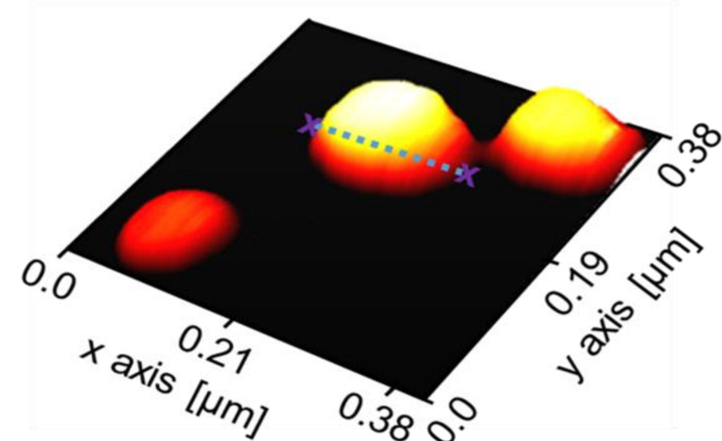
Characteristic	CTRL, N = 19[1]	HCC, N = 20[1]	p-value[2]
Age (years)	65.8 (4.8)	68.7 (5.9)	0.14
Gender			0.7
F	26%	20%	
M	74%	80%	
Cholesterol (mg/dl)	180 (19)	170 (46)	0.5
Triglycerides (mg/dl)	122 (38)	101 (30)	0.023
GPT-ALT (UI/L)	15 (3)	45 (23)	<0.001
GOT-AST (UI/L)	20 (5)	47 (21)	<0.001
AST/ALT	1.43 (0.53)	3.86 (11.37)	0.3

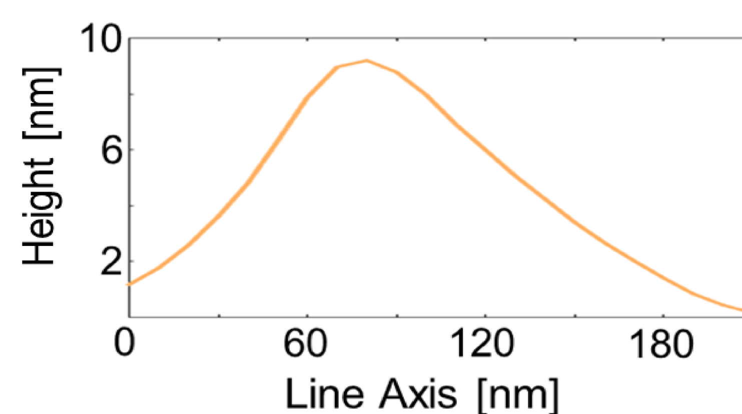
Characteristic	CTRL, N = 19[1]	HCC, N = 20[1]	p-value[2]
GGT (UI/L)	31 (5)	179 (147)	<0.001
ALP (UI/L)	171 (17)	272 (137)	0.049
Hb (mmol/L)	14.32 (0.96)	12.78 (2.78)	0.15
Creatinine (mg/dL)	0.79 (0.18)	1.42 (1.29)	0.071
Azotemia (mg/dL)	15 (3)	21 (10)	0.067
Bilirubin (mg/dl)	0.69 (0.23)	1.63 (1.04)	<0.001
PIVKA	14 (4)	3,205 (9,987)	<0.001
AFP	2 (1)	193 (417)	0.001

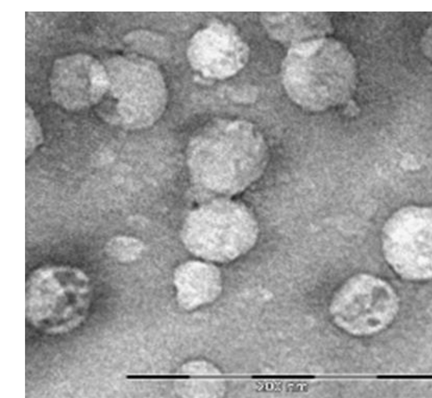
[1] Mean(SD);%
[2] Wilcoxon rank sum test; Fisher's exact test.

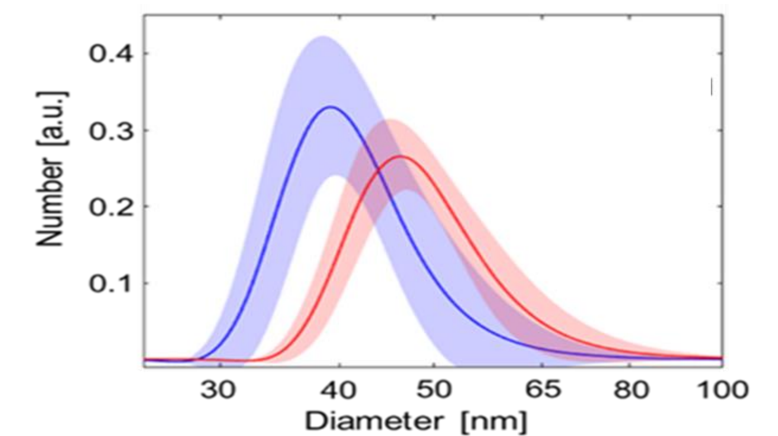
To study the different cell phenotype, we treated Caco-2 cells with N-acetyl-L-cysteine (NAC). The assessment of NAC-induced cell differentiation was obtained by electron and confocal fluorescence microscopy (left panels). The MET transition was estimated by assessing the expression levels of E-cadherin protein at cell junctions (AlexaFluor-488, green). SEM micrographs show the morphological change from mesenchymal to epithelial phenotype, while the TEM insert allows visualization of epithelial microvilli.
The blood samples of the subjects enrolled for the study were characterized by biochemical parameters to know their condition. A summary of the clinical parameters is given in the tables (right panels).

Small Extracellular Vesicles isolation and characterization





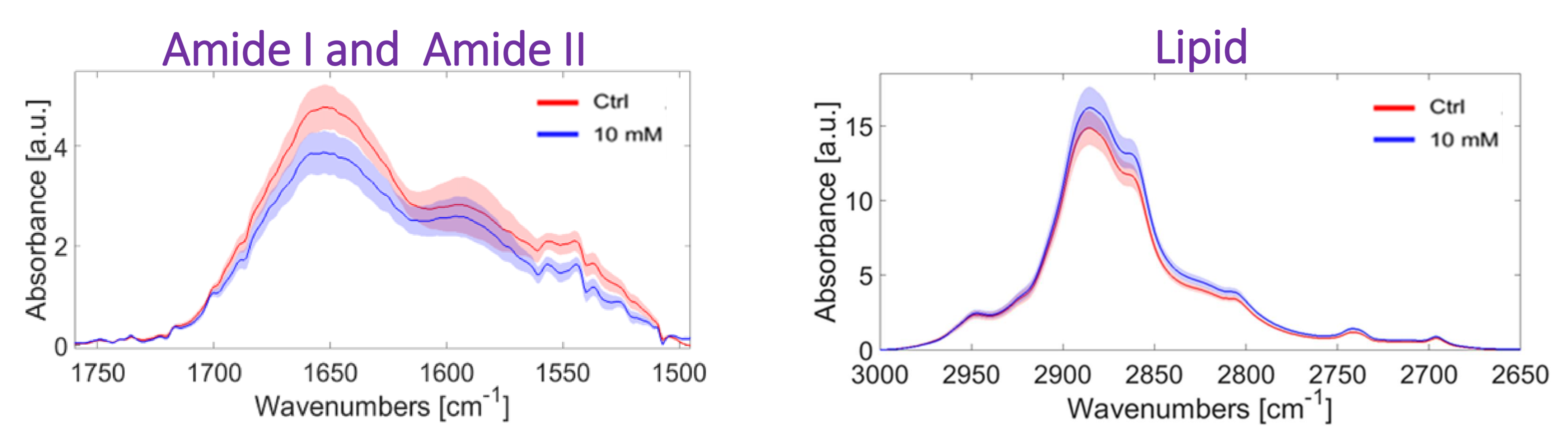


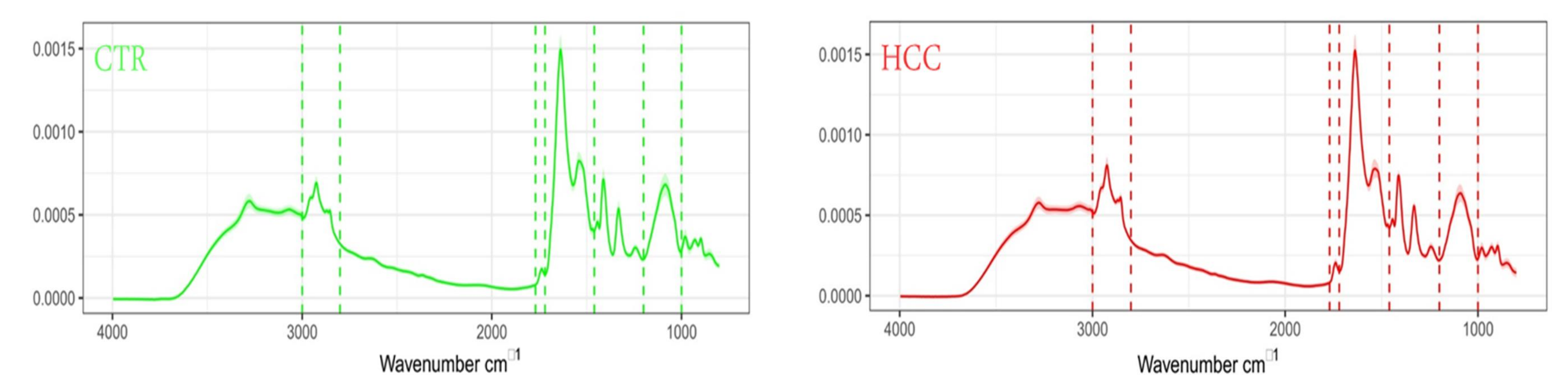


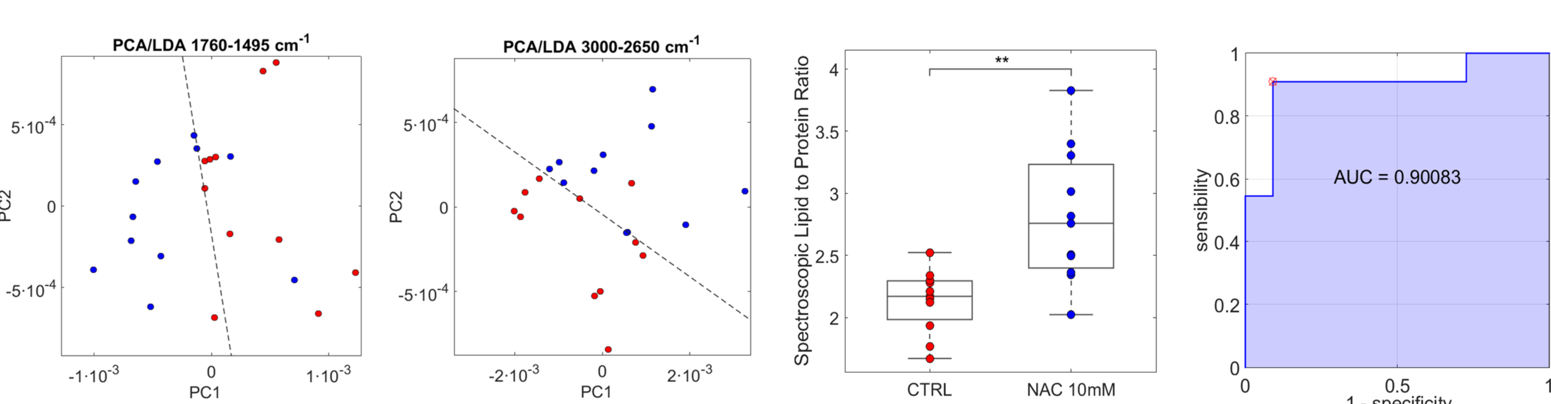
Isolation of EVs was achieved using the ExoQuick-TC precipitation kit for cell culture medium and ExoQuick-Ultra for subject serum, respectively. Evaluation of morphological characteristics and size distribution was performed using atomic force microscopy, transmission electron microscopy and dynamic light scattering. The representative images shown above are consistent with the isolation of small extracellular vesicles (SEVs).

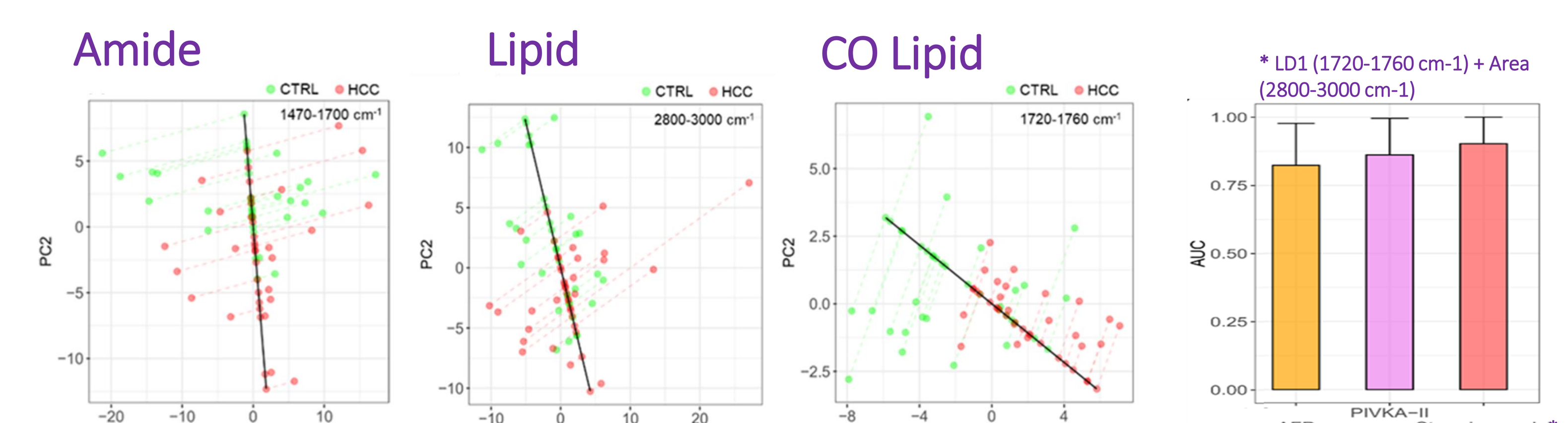
FT-IR assisted classification of Small Extracellular Vesicles from different origin

The FTIR absorbance spectra of the two cell phenotypes were obtained by averaging the measurements corresponding to the 11 different independent replicates of the experiment and the subsequent SEVs extractions (11 CTRL, 11 NAC). The most informative bands [1,2], i.e. the 1700-1500 cm^{-1} absorption bands of amides I and II and the 3000-2700 cm^{-1} absorption band of lipids, were reported in the left panels correlated by the PCA-LDA statistical approach and the ROC curve.
For the clinical study[3], FTIR spectra were obtained by averaging 20 SEVs measurements corresponding to 20 different independent subjects (19 CTRL, 20 HCC). The complete spectra are shown in the right panels. The dot lines highlight the most informative bands correlated by PCA-LDA, Stepwise logistic regression and ROC curve.









In conclusion, we have demonstrated that mid-ir spectroscopy is a powerful tool for the label-free biochemical characterization of EVs with potential diagnostic applications. We have shown that the label-free spectral approach can automatically classify small extracellular vesicles derived from human cancer cell lines with different phenotypes, with good classification capability. We demonstrate the concept of the liquid biopsy approach for HCC diagnosis and reveal the potential positive impact on the development of a new diagnostic tool.

REFERENCES

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