A neutrophil metabolic switch in patients with Hepatocellular Carcinoma

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Introduction

Chronic inflammation is a common trait sustaining the development/progression of 90% of hepatocellular carcinomas (HCC). Neutrophils have emerged as key immune mediators involved in promoting cancer progression/metastases. Our group has previously demonstrated that neutrophil depletion in a murine HCC model has profound anti-tumour effects [1], whilst peripheral circulating neutrophil count was an independent predictor of patient poorer survival [2]. Pan-neutrophil depletion is not a viable option for cancer patients already susceptible to infection; however, distinctive cancer-specific markers and functions are still poorly defined.

AIM: To explore distinct immunophenotypic features of peripheral blood neutrophils in an effort to identify potential biomarkers linked to the development/progression of HCC.

Methods

Neutrophil heterogeneity was assessed between liver cirrhosis and HCC patients (Newcastle, 2016-2019), together with healthy volunteers (n = 25, 81, and 47, respectively). Circulating neutrophil phenotype and apoptosis were evaluated by flow cytometry, including 10 surface markers, and mass spectrometry proteomics. Functional assays assessed respiratory burst, comparing basal and stimulated levels of reactive oxygen species (ROS) in response to N-formyl-Met-Leu-Phe (fMLP) plus platelet-activating factor (PAF), and phorbol 12-myristate 13-acetate (PMA), and metabolism (Seahorse Analyser).

Results 1

Neutrophils present an immature phenotype

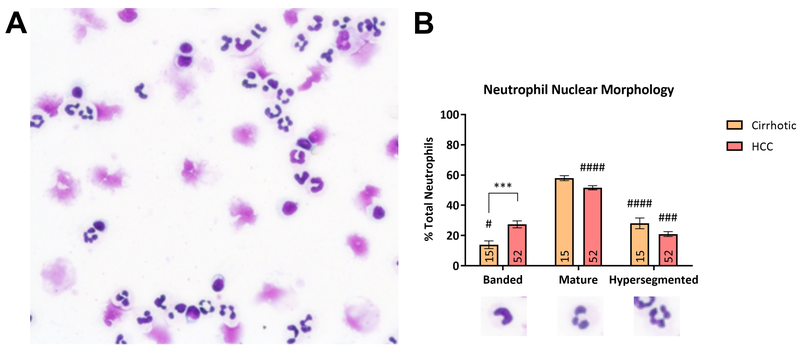

Fig 1. (A) Representative image of cytospin stained with Giemsa. (B) Graph summarising average percentages of banded, mature and hypersegmented neutrophils. # denotes differences versus healthy controls.

Results 2

There are less apoptotic neutrophils in circulation, and these have a dysfunctional immunophenotype seen by CD11b and CD62L surface levels

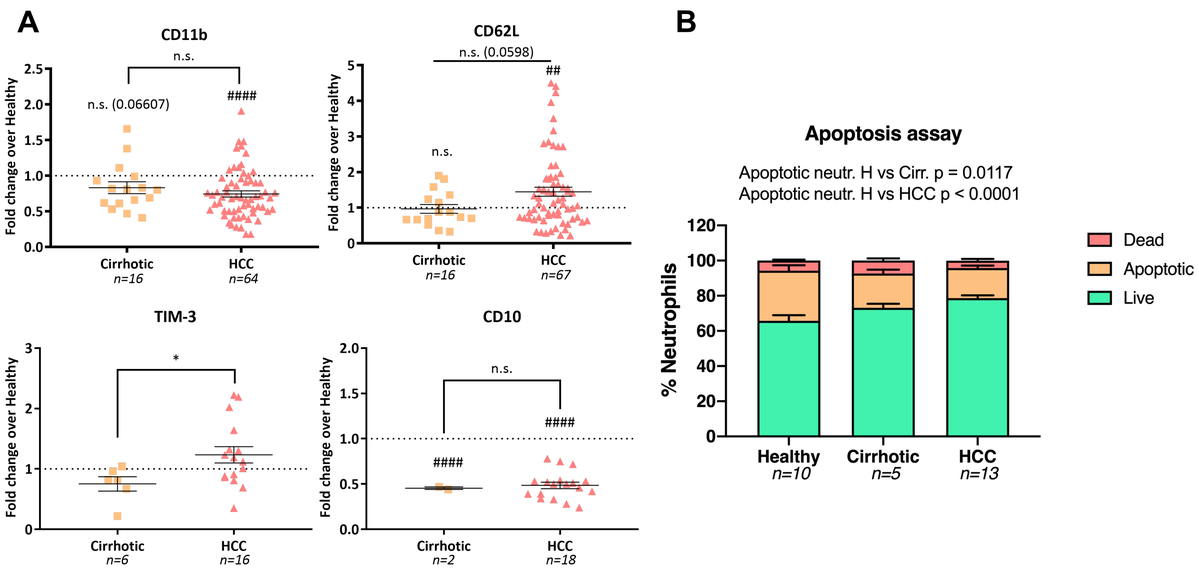

Fig. 2. (A) Surface marker expression of circulating neutrophils from cirrhotic and HCC patients normalised to healthy controls (# denotes differences versus healthy controls). (B) Percentage of live (Annexin V-/PI-), apoptotic (Annexin V+/PI-) and dead (Annexin V-/PI+ and Annexin V+/PI+) neutrophils from healthy controls and cirrhotic/HCC patients (mean ± SEM). Significant differences are denoted by p-values above the graph.

Results 3

Neutrophils present an impaired respiratory burst capacity when stimulated with PAF + fMLP, but a greater one when stimulated with PMA

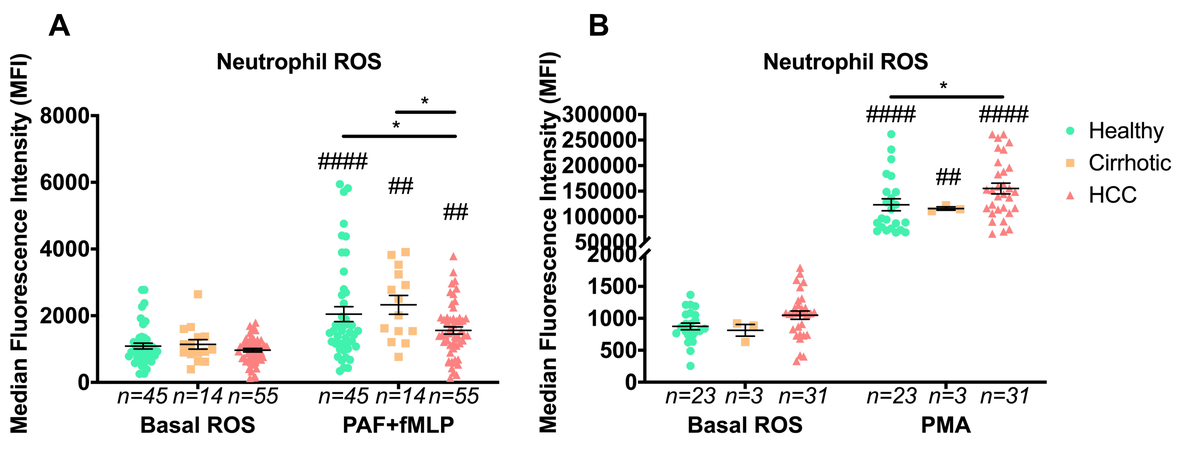

Fig. 3. Basal and stimulated ROS production by circulating neutrophils following (A) PAF and fMLP, and (B) PMA (mean ± SEM). # denotes differences versus basal levels.

Results 4

Neutrophils undergo a metabolic switch toward mitochondria oxidative phosphorylation

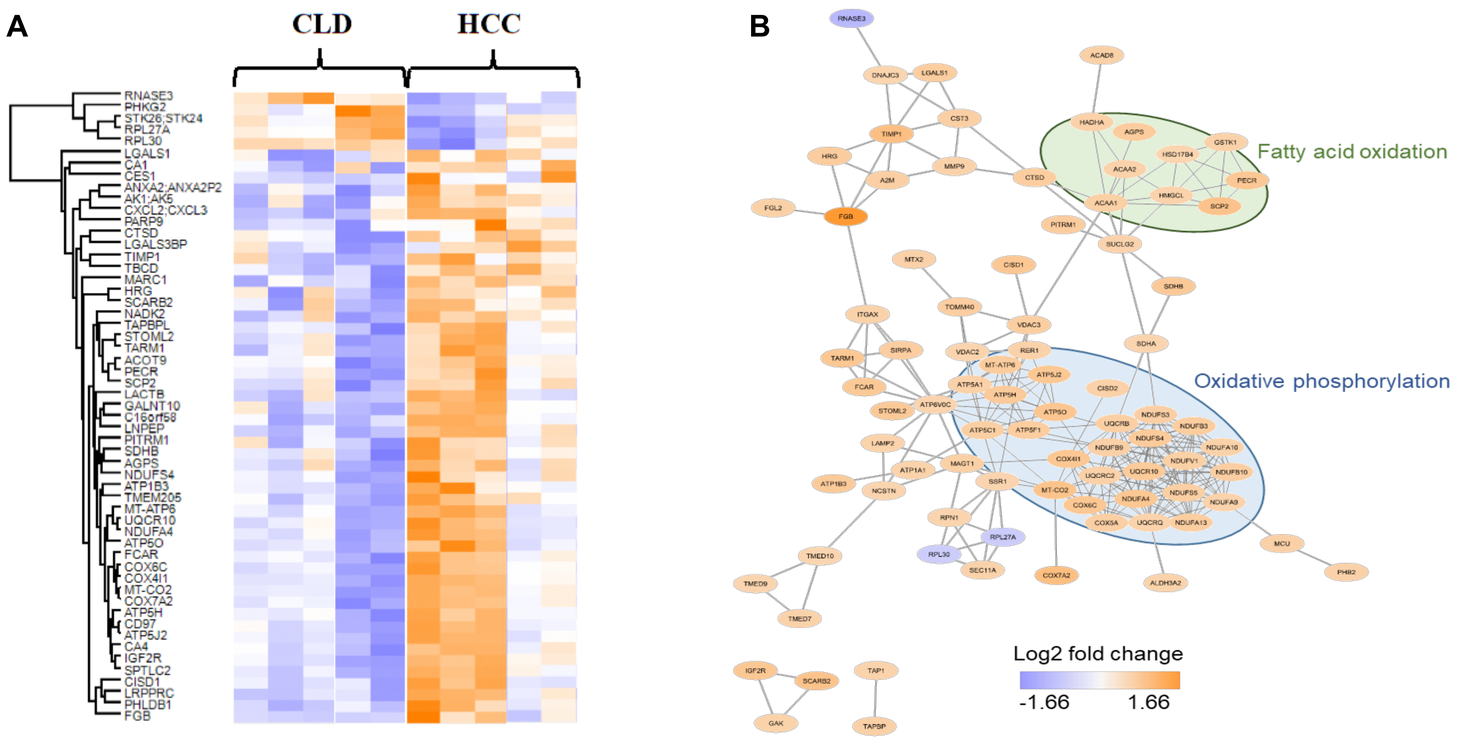

Fig. 4. (A&B) Heat-map and STRING network analysis based on mass spectrometry proteomics of circulating neutrophils. (C) Seahorse metabolic flux analysis of circulating neutrophils (OCR, Oxygen Consumption Rate; a. glucose, b. oligomycin, c. FCCP, d. rotenone/antimycin A). (D) Graphical representation of the mechanistic hypothesis.

Conclusion

In conclusion, this data suggests that there are phenotypic and biologically relevant changes in peripheral blood neutrophils from liver cancer patients, and that further characterisation of these cells may enable us to better define the “pro-tumour neutrophil” associated with HCC and develop potential tailored therapeutics to target these cells selectively.

References

[1] Wilson, C.L., et al. NFkappaB1 is a suppressor of neutrophil-driven hepatocellular carcinoma. Nature communications 6, 6818 (2015).

[2] Margetts J et al. Neutrophils: driving progression and poor prognosis in hepatocellular carcinoma? British Journal of Cancer 118(2):248-257 (2017).