**Platelet-derived microparticles of obese individuals induce endothelial-to-mesenchymal transition and produce higher levels of thromboxane A2: a possible mechanism in obesity-driven tumorigenesis**

1)Dovizio M.. 2)Grande R.. 3)Fullone R.. 4)Schiavone S.. 5)Tacconelli S.. 6)Marchisio M.. 7)Lanuti P.. 8)Caprodossi A.. 9)Mingrone G.. 10)Patrignani P..

*"G. d'Annunzio" University of Chieti*

Microparticles (MPs) are shed by cells upon activation and are recognized as conveyors of intercellular communication through the transmission of biological signals(1). The 70%-90% of plasma MPs are platelet-derived(2). Numerous lines of evidence support the link between excess body weight and increased risk of developing and dying from several types of cancer(3). However, the biological mechanisms underlying this relationship have not been elucidated yet. We have hypothesized that platelet-derived MPs(PMPs) play a role in cancer development and progression by inducing endothelial-mesenchymal transitions(EndMT) which can participate in tumor progression by providing cancer-associated fibroblasts(4).

The first objective of this study was to characterize PMPs isolated from obese individuals versus healthy subjects for their capacity to generate lipid mediators, such as prostaglandin(PG)E2 and thromboxane(TX)A2, and for the expression of CD61, a platelet receptor subunit of glycoprotein IIb/IIIa(CD41/CD61) on their surface. The second objective was to compare the capacity of PMPs, from obese and healthy subjects, to induce EndMT in vitro.

MPs (250 MPs/□l), isolated from thrombin-stimulated platelets(5), were cultured alone or with normal Human Microvascular Endothelial Cells(HMVEC, 2x105 cells) up to 24h. In the conditioned medium, the levels of prostanoids(such as PGE2 and TXB2, the hydrolysis product of TXA2) were assessed by validated immunoassays(6). PMPs, from healthy volunteers cultured alone, released TXB2 in a time-dependent fashion [617.5±93.2 pg/ml (at 0h), 834.1±72.81 pg/ml (at 2h), 1905.0±436.3 pg/ml (at 4h) and 2073.0±360.0 pg/ml (at 24h), mean±SEM, n=3], while the levels of PGE2 were undetectable. Western Blot analysis of PMPs showed that they contain all the enzymatic machinery necessary for the biosynthesis of TXA2. The levels of TXB2 released from platelet MPs isolated from obese individuals and cultured for 24h were significantly higher than those released from PMPs of healthy subjects (3779.0±793.2 pg/ml and 2047.0±593.7, respectively, $P<0.01$, mean±SEM, n=4). In obese individuals, differently from healthy subjects, the levels of TXB2 released from PMPs positively correlated with the expression of CD61 on the MP surface ($r2=0.795$; $P<0.05$). PMPs isolated from obese individuals released a higher amount of TXB2 even in the co-culture with HMVEC cells for 24h, versus those produced by PMPs isolated from healthy subjects (2690.0±382.7 pg/ml and 910.1± 59.4 pg/ml, respectively, $P<0.05$). In the co-culture of HMVEC with PMPs of obese individuals, the expression of the pro-inflammatory gene COX-2 (1.32±0.10 fold change, $P<0.05$) was induced. Moreover, EndMT occurred as shown by the downregulation of VE-cadherin (endothelial marker, 0.71±0.06 fold change, $P<0.05$) and upregulation of □-smooth muscle actin (SMA) (a mesenchymal marker, 1.40±0.04 fold change,

P<0.01) versus endothelial cells cultured alone. Differently, in the co-cultures of HMVEC with PMPs from healthy subjects, only □-SMA was significantly induced versus untreated endothelial cells.

In conclusion, our findings show that platelet MPs from obese individuals produce higher levels of TXA2, a potent stimulus for platelets activation, vascular SM cell proliferation, and angiogenesis, versus MPs of healthy subjects. Moreover, platelet MPs of obese individuals have a higher capacity to induce EndMT transition in microvascular endothelial cells than MPs from healthy subjects. These effects might participate in obesity-driven cancer. Since the antiplatelet agent low-dose aspirin can curb platelet MP generation(7), our results open the way to perform further studies to verify whether the drug plays an antitumorigenic effect by preventing MP-dependent EndMT induction.

References

1. Hargett et al. Pulm Circ. 2013;3:329-40

2. Burnouf et al. Blood Rev. 2014;28:155-66

3. Kyrgiou et al. BMJ. 2017 Feb 28;356:j477

4. Zeisberg et al. Cancer Res. 2007;67:10123-8

5. 24. Vasina et al. Am J Blood Res. 2013;3:107-23

6. Patrignani et al. JPET 1994;271:1705-12

7. Giacomazzi et al. Front Pharmacol. 2016 16;7:314