In vitro VPA-differentiated megakaryocytes express functionally active Tissue Factor which is delivered to the nascent platelets

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Introduction?Tissue factor (TF), a 47kDa transmembrane glycoprotein, is the main activator of the blood coagulation cascade. At the beginning of the 2000 it was shown that also platelets, among the blood circulating cells, could be a source of TF which can sustain activation of coagulation on the edge of a growing thrombus. Since then, different research groups, both in Europe as well as in the USA, confirmed the presence of functionally active TF in platelets. The main mechanism claimed to be responsible for the presence of TF within platelets is through the uptake of TF positive microparticles released by activated monocytes or endothelial cells. It should be considered however that platelets, derived from megakaryocytes through an highly regulated fragmentation process, are released into the blood stream with a selection of proteins and mRNAs. **Aim** ? In the present study we tested the hypothesis that megakaryocytes express both the TF mRNA and protein, which are then delivered to the nascent platelets. To this aim we took advantage from the use of a human megakaryoblastic cell line, MEG-01, able to differentiate into polyploid megakaryocytes (MK) releasing platelets. **Methods** ? MEG-01, cultured in RPMI with 10%FCS and antibiotics were differentiated into megakaryocytes with valproic acid (VPA, 2mM). MK and platelet-like particles (PLPs) released into the culture medium were characterized for the presence of specific population markers (glycoprotein IIb [CD41] and IIIa [CD61]) as well as for TF protein expression by ELISA, flow cytometry and immunocytochemistry. TF mRNA was assessed by RT-PCR both in MK as well as in PLPs. **Results** ? VPA-induced megakaryocytic differentiation resulted in a significant increase in the percentage of adherent cells (from 2,6±1,5 to 70±2,3) which showed the typical feature of MK such as polyploidy, formation of proplatelets and elevated expression of the MK marker CD41 and CD61 (90% and 95%, respectively). Also PLPs were CD61+ (65%) and CD41+ (60%). TF protein levels was two times greater in MK than in megakaryoblasts (0,05±0,01 and 0,09±0,02 respectively), and the protein was also three times more active in MK than in megakaryoblasts (4±1,8 and 14±7 respectively). Also PLPs expressed TF active protein (0,32±0,08). Flow cytometry analysis confirmed that MK express more TF than megakaryoblasts (75% positive cells VS 45%) and only 40% of PLPs released by MK are TF positive. RT-PCR experiments confirmed the presence of TF as well as of GpIIIa, GpIb and COX1 mRNA both in MK and in PLPs. In particular MK showed a 3-fold increase of TF mRNA compared to megakaryoblasts. **Conclusions** ? The use of the well characterized human megakaryoblastic cell line MEG-01 allowed us to show the presence of TF mRNA and protein in MK. TF expression increases during differentiation from megakaryoblast to megakaryocyte. Moreover we provide for the first time the evidence that megakaryocytes are able to transfer TF mRNA and protein to the released platelets supporting the hypothesis that the megakaryocyte-transfer mechanism may account for the presence of TF in platelets.