

treated with healthy subjects MPs did not affect flow-induced dilation, these vessels showed a reduced prostacyclin-component that was completely compensated by the NO-component of the response. The endothelial dysfunction induced by MPs from OSAS was caused by the reduction of both NO- and prostacyclin- but not the endothelium-derived hyperpolarizing factor-components of the response in SMA. These data provide evidence that circulating MPs from OSAS patients influence both endothelial function and vascular reactivity.

E007

DIFFERENTIAL EFFECTS OF MICROPARTICLES FROM HUMAN APOPTOTIC T LYMPHOCYTES AND FROM HUMAN APOPTOTIC MONOCYTES IN ENDOTHELIAL CELLS

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During cell activation or apoptosis, cells release vesicles, also called microparticles (MPs) from the plasma membrane. Since composition of MPs is dependent on cell origin and the type of stimulation, we compared the effects of MPs generated from both apoptotic T lymphocytes and apoptotic monocytes on endothelial function with respect to both nitric oxide (NO) pathway and reactive oxygen species (ROS). MPs were produced by treatment of either human T lymphocytes with the apoptotic agent actinomycin D or human monocytic cell line THP-1 with the apoptotic agent, the etoposide VP-16. Human Eahy 926 endothelial cells were incubated with 10 µg/ml MPs for 24h. Apoptotic MPs from human T lymphocytes decreased NO production that was associated with overexpression and phosphorylation of endothelial NO-synthase (eNOS). Also, T lymphocytes MPs enhanced expression of caveolin-1 and decreased its phosphorylation. T lymphocytes MPs enhanced ROS by a mechanism sensitive to xanthine oxidase and P-IkappaBalpha inhibitors. PI3-kinase inhibition reduced the effects of T lymphocytes MPs on eNOS, but not on caveolin-1, whereas it enhanced the effects of MPs on ROS production. Inhibition of MEK reversed eNOS phosphorylation but it had no effect on ROS production induced by T lymphocytes MPs. By contrast, apoptotic MPs from human monocytes increased both NO production and in much less extent ROS. These effects were associated with a decrease of caveolin-1 expression and increased its phosphorylation, without affecting eNOS expression and phosphorylation. The inhibitor of the PI-3kinase, LY294002, reversed the effects of monocyte MPs on caveolin-1 expression but not on its phosphorylation. The MEK1/2 inhibitor, U0126, reversed the decrease of caveolin-1 expression induced by MPs from monocytes. Interestingly, U0126 potentiated ROS production induced by monocyte MPs. Whereas in vivo injection of T lymphocytes MPs in mice impaired endothelial function, apoptotic MPs from human monocytes did not affect endothelium-dependent relaxation. In addition, monocyte MPs were able to promote in vitro angiogenesis. In summary, these results highlight differential effects of apoptotic MPs from different origins by activating diverse multiple pathways related to NO and ROS productions.

E008

STUDY ON THE MECHANISM OF HUMAN TELOMERASE ASSEMBLY

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Telomerase, a ribonucleoprotein enzyme, catalyses the synthesis of repeated telomeric sequences. Telomeres are required for chromosome stability and cell viability and a decrease of their length was found to be associated with several cardiovascular diseases. Telomerase contains the hTR RNA, and several proteins, including the reverse transcriptase hTERT. The hTR 5' domain contains the template sequence for DNA synthesis and binds hTERT. The H/ACA 3' domain is required for stability and associates with 4 proteins (Dyskerin, GAR1, NOP10 and NHP2 proteins). Limited information is available on telomerase assembly. A pre-complex containing Dyskerine, NOP10, NHP2 and an assembly factor, called NAF1, is likely formed in the cytoplasm and transported to the nucleus, where it associates with the H/ACA domain of nascent RNA. Exchange of NAF1 for GAR1 allows the production of the functional H/ACA RNP domain. We showed that several factors are implicated in H/ACA RNP assembly : NUFIP, the R2TP complex, and HSP90. The SMN complex, containing the SMN, Gemin2 to Gemin8 and Unrip proteins may also be involved, since protein SMN interacts with GAR1.

For further analysis on the role of the SMN complex in telomerase assembly, we tested by yeast two hybrid assays whether other proteins of this complex can interact with H/ACA RNP proteins and their assembly factors. We discovered a possible interaction between NAF1, Gemin 3 and Gemin8. Hence, the SMN complex may be involved in the replacement of NAF1 by GAR1 : it may associate with GAR1 through an interaction with SMN, and with the pre-H/ACA RNP complex through interaction of NAF1 with Gemin3 and 8, allowing the replacement of NAF1 by GAR1. We are testing this hypothesis. The SMN complex may also be involved in hTERT binding to the 5' domain of hTR and we will also explore this possibility.

Through these studies we should bring insight into telomerase assembly that may be useful to understand telomere attrition in cardiovascular diseases.

E009

CARACTERISATION DES MICROPARTICULES CIRCULANTES CHEZ LES PATIENTS ATTEINTS DU SYNDROME DES ANTICORPS ANTI-PHOSPHOLIPIDES

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Contexte – Les MP sont générées par le remaniement de la membrane plasmique suite à l'activation des cellules sanguines ou des cellules endothéliales.

Objectif – Nous avons caractérisé les microparticules circulantes chez les patients atteints du syndrome des anticorps anti-phospholipides (SAPL), thrombophilie acquise caractérisée par un risque augmenté de thromboses et de complications obstétricales.