Metabolism and cancer: the CD38-NAMPT connection in chronic lymphocytic leukemia

Valentina Audrito[1,2], Tiziana Vaisitti[1,2], Sara Serra[1,2], Davide Rossi[3], Giovanni D'Arena[4], Luca Laurenti[5], Gianluca Gaidano[3], Fabio Malavasi[1] and Silvia Deaglio[1,2]

[1] Department of Genetics, Biology and Biochemistry, University of Torino Medical School, 10126 Torino, Italy; [2]Human Genetics Foundation (HuGeF), 10126 Torino, Italy; [3]Division of Hematology, Department of Clinical and Experimental Medicine & BRMA, "Amedeo Avogadro" University of Eastern Piedmont and AOU Maggiore della Carità, 28100 Novara, Italy; [4]IRCCS "CROB", Rionero in Vulture, Italy; [5]Institute of Hematology, Catholic University of the Sacred Heart, Rome, Italy.

E-mail contact: valentina.audrito@unito.it

Tumor transformation is generally accompanied by an altered metabolic state, with higher needs for NAD, an essential co-factor in the oxidative phosphorylation chain, as well as a substrate for four classes of enzymes, including the NADase CD38. In leukocytes, NAD is synthesized mainly from nicotinamide through the activity of nicotinamide phosphoribosyl transferase (NAMPT), which is the first and rate-limiting enzyme in this biosynthesis pathway. In addition to its intracellular localization (iNAMPT form), the enzyme can be present extracellularly (eNAMPT), where it exerts cytokine-like actions that promote the maturation of early stage B cells. For this reason it is also known as pre-B cell colony enhancing factor (PBEF).

Our hypothesis is that PBEF/Nampt exerts pro-survival activity in human leukemic B cells through the generation of a pro-inflammatory microenvironment. The model selected is chronic lymphocytic leukemia (CLL), a disease characterized by the slowly progressive expansion of mature $CD5^+$ B lymphocytes, intrinsically resistant to apoptosis and dependent on a growth supportive environment for progression. A further reason for selecting this model is that CD38 is an independent negative prognostic marker for CLL patients, suggesting that modulation of the extracellular NAD/nicotinamide balance is critical in determining a more aggressive phenotype.

Analysis of the expression of i and eNAMPT shows that CLL cells (n=60) express high and homogeneous levels of iNAMPT comparable with those scored by normal B lymphocytes from peripheral blood of health donors. In contrast, plasma eNAMPT levels are significantly higher (fourfold increased, p=0.001) in CLL patients (n=50) when compared to controls. These data suggest an involvement of eNAMPT in this disease, also confirmed by *in vitro* experiments where CLL cells, cultured in the presence of recombinant eNAMPT, showed activation and proliferation. Furthermore, after 5 days of treatment with eNAMPT CLL cells displayed morphological features of immunoblasts, as observed after activation of CD38 using a combination of agonistic mAbs and IL-2. In line with our hypothesis of a direct interplay between CD38 and eNAMPT, i) $CD38^+$ CLL cells were selectively responsive to eNAMPT actions and ii) CD38 activation led to a potent and reproducible increase in eNAMPT. Combined treatment of CLL cells with agonist anti-CD38 and IL2 was followed by i) a rapid increased of NAMPT mRNA and ii) a marked secretion of eNAMPT, present in culture supernatants after 5 days of treatment. No difference was observed in iNAMPT levels, constantly elevated.

Considered together, these data suggest the existence of a CD38/eNAMPT extracellular loop, where CD38 consumes NAD and generates nicotinamide, triggering eNAMPT expression and activation to reconstitute extracellular NAD levels. This loop appears to be operative in CLL cells, generating pro-survival and activation signals.