209 Nicotinamide acts as pharmacological agent by promoting apoptosis in chronic lymphocytic leukemia through the activation of the p53/miR-34a/SIRT1 tumour suppressor network

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Nicotinamide (Nam), the amide form of niacin, is the main precursor of nicotinamide adenine dinucleotide (NAD^+). It regulates availability of this co-enzyme and consequently activity of NAD^+-consuming enzymes. Interest in Nam has recently been refueled by data showing its activity as anti-inflammatory and anti-oxidant agent. Nam is also reported to exert potent inhibitory effects on murine B lymphocytes.

The aim of this work was to extend the observations obtained in mice to human models and to compare the effects of exogenous Nam to life/death economy of normal *vs.* leukemic B lymphocytes. Chronic lymphocytic leukemia (CLL) was selected as a disease model due to its intrinsic clinical-biological features.

The results show that pharmacological doses of Nam (5−10 mM) significantly inhibit proliferation and induce apoptosis of CLL cells. At earlier time points, Nam markedly reduces phosphorylation of multiple intracellular substrates, including the MAPK family member ERK1/2. Normal B lymphocytes, used as control, were significantly less sensitive to the action of Nam. A hypothesis to explain the above data is that Nam exposure blocks the activity of NAD^+-dependent enzymes. Attention was focused on SIRT1, a deacetylase that plays a critical role in cancer and that acts as a longevity factor. Nam exposure blocks the activity – and also the expression – of SIRT1 from nuclear extracts of CLL cells, but not of normal B lymphocytes, obtained from spleen or tonsils. SIRT1 is a negative regulator of p53 expression and its function is to decrease p53-mediated apoptosis following DNA damage. Our hypothesis is that the link between these two molecules is represented by miR-34a, a direct transcriptional target of p53 that can bind SIRT1 mRNA and trigger its degradation. In line with this hypothesis, a combined treatment of CLL cells with Nam and etoposide (a DNA-damaging chemotherapeutic) was followed by (i) up-regulation of miR-34a expression, (ii) marked down-modulation of SIRT1 expression and function, and (iii) induction of expression and acetylation of p53. This positive feedback loop was operative in the CLL model, with the final outcome of a tumour cell apoptosis significantly enhanced.

These data indicate that CLL cells are uniquely sensitive to the pharmacological actions of Nam and prompt further studies to evaluate whether this vitamin can be added to the armamentarium of therapies for CLL patients, due to a well-established safety profile.

210 Identification and characterization of amphiregulin as a new biomarker of resistance to gefitinib in non-small cell lung cancers

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Background: Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancers and is associated with a very poor prognosis, with a 5-year survival rate remaining below 15%. Gefitinib is a molecule that belongs to Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors (EGFR-TKIs) family and has shown potent antiproliferative effects in NSCLC. A high variability in clinical responses to this treatment lead to investigate new predictive markers to discriminate gefitinib-responders from non-responders. Patients that are resistant to gefitinib have high seric amphiregulin (AREG) levels, suggesting a role of this growth factor in gefitinib resistance. We investigated whether AREG was involved in gefitinib resistance and characterized the molecular pathway initiated by AREG.

Material and Methods: First, we analysed gefitinib resistance *in vitro* in two NSCLC cell lines, the AREG-secreting H358 cells and the AREG non secreting H322 cells. We used anti-AREG siRNAs to silence AREG expression in H358 cells and we add recombinant human AREG to H322 cells. Co-immunoprecipitations, apoptosis assays and transfections allowed the complete characterisation of the mechanism of resistance. Then we performed *in vivo* tumour xenografts in nude mice to confirm to role of AREG in the resistance and investigate alternative treatments.

Results: We showed that AREG allows resistance to gefitinib-induced apoptosis *in vitro* and *in vivo*, through the inactivation of Bax proapoptotic protein. AREG induces the decrease of Bax expression level as well as an increased interaction between Bax and Ku70, through an acetylation-dependant mechanism. Therefore we described an original pathway of gefitinib resistance, dependant of acetylation, and controlled by the growth factor AREG. We finally observed an *in vivo* synergistic activity between gefitinib and vorinostat, a histone deacetylase inibitor.

Conclusions: Lung cancer is a major health problem and NSCLC resistance to treatments represents a worrying phenomenon for clinicians. This work suggests both diagnostic and therapeutic solutions to improve NSCLC care. Effectively, we demonstrated the major role of AREG in NSCLC resistance to gefitinib, and validated its use as a predictive biomarker of non responsiveness to this treatment, as well as a therapeutic target. Moreover, we uncovered an original pathway leading to resistance to EGFR-TKIs suggesting the interest to associate EGFR-TKIs with histone deacetylase inhibitors, especially for NSCLC patients that are resistant to EGFR-TKI.

211 Profiling cancer related kinases using a universal ADP detection platform

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Because the selectivity of kinase inhibitor towards the target of interest is critical for its advancement through the various phases of cancer drug development, profiling of the inhibitor against other kinases is a common practice in drug discovery. Thus, a robust and universal kinase assay is desired in order to assess the selectivity and potency of the inhibitor on multiple kinases from different classes that often use substrates with different chemical structures. Radiometric assays have been widely accepted as the most reliable platform for profiling kinases. Although several other techniques were developed in the last few years, most suffer from a variety of limitations that makes it difficult to address all teh needs of kinase screening, mode of action (MOA) studies and profiling with a single platform during the drug discovery process. ADP-Glo(tm) technology measures kinase activity by quantifying the amount of ADP produced during the reaction. This assay is universal, applicable to all kinds of kinase substrates regardless of their nature with no prior modification (peptides, proteins, alcohols, lipids, and sugars), making it easier to create inhibitor profiles of different kinase families using one platform. The assay is as sensitive as a radiometric method but without the use of radioactivity and disposal of radioactive waste. Because of the high signal to background (S/B) values even at low % ATP to ADP conversion, the assay allows significant savings of enzyme usage in kinase assays. The fact that the ADP-Glo assay offers so many positive attributes makes it an ideal assay for primary and secondary screening but also for profiling lead compounds.

212 The a5β1 integrin is a therapeutic target for human glioblastoma and participates to chemoresistance

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Background: Of solid tumours, glioblastoma remains the most resistant to therapy. Despite advances in neurosurgery, radiation and medical oncology, the prognosis for patient with glioblastoma did not improve in the last 30 years. A better molecular and biological knowledge of glioma will lead to advances for the management of glioblastoma. Integrins were recently identified as potential therapeutic targets as they take part in various cancer stages such as malignant transformation, tumour growth and progression, invasion and metastasis and angiogenesis. The specific role of $\alpha5\beta1$ integrins in cancer has recently attracted much interest. We investigated its role in human glioblastoma.

Methods: Expression levels of $\alpha_5\beta_1$ integrin were analyzed in 94 adult glioma patient samples by qPCR and compared to normal brain. Glioblastoma cell lines (U87MG and U373) were silenced ($\alpha5_{\ low}$) or forced to express ($\alpha5_{\ high}$) the $\alpha5$ subunit of the integrin. Protein expression and activity was determined by western blots. Drugs used were SJ749 and K34c ($\alpha_5\beta_1$ integrin antagonists), LY294002 (an inhibitor of PI3K) and Temozolomide (TMZ). Surviving fraction after drug treatment was determined by clonogenic assays.

Results: $\alpha5\beta1$ integrin expression in patient biopsies correlates with the tumour grade. High expression levels of $\alpha5\beta1$ integrin were detected in 71% of adult glioblastoma as compared to 14% and 34% respectively in grade II and grade III glioma samples. $\alpha5_{\ high}$-cells were resistant and $\alpha5_{\ low}$-cells were highly sensitive to TMZ as compared to mock-transfected cells. Concomitant treatment of $\alpha5_{\ high}$-cells with integrin antagonists and Temozolomide restored the cell sensitivity to TMZ. PI3K/AKT pathway was similarly significantly reduced by decreasing $\alpha5$ expression or by using integrin antagonists. Additionally, a5 integrin subunit expression level inversely modulates p53 activity and the induction of p53 target genes. In $\alpha5_{\ low}$-cells, TMZ-induced p53 activity was increased and inversely, in $\alpha5_{\ high}$-cells TMZ-induced p53 activity was significantly decreased. Therefore we postulate that $\alpha5_{\ high}$-cells are less sensitive to TMZ therapy through modulation of PI3K/AKT and p53 signaling pathways.

Conclusions: The $\alpha5\beta1$ integrin is a marker of glioblastoma aggressiveness and modulates glioblastoma chemosensitivity towards Temozolomide. Specific antagonists may be potent adjuvant to chemotherapy in the subpopulation of patients expressing high levels of $\alpha5\beta1$ integrin.