Material and methods: For stable transfections MDCK cultured cells were transfected with the pFLAG-MDGA1 construct or the empty pFLAG vector. Transfected cells were selected by growing in DMEM containing 10% fetal calf serum and 500 μg/ml of G418 for 3 weeks. Resistant clones were isolated and MDGA1 expression was analysed by Western blotting. Then MDCK cells expressing MDGA1 were used in cell adhesion assays, invasion assays and cell migration assays.
Results and conclusion: In order to investigate a potential implication of MDGA1 in cellular adhesion, attachment of cultured cells to several ECM proteins such as, collagen type I and IV, fibronectin and laminin was assessed. Moreover, cell invasion was carried out using a modified Boyden chamber assay and finally, MDCK cell motility was assessed using a scratch wound assay. Results seem to indicate that MDGA1 has a functional role related to adhesiveness and motility.

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Sensitivity of single, *in vitro* growing lung cancer cells to gemcitabine measured with synchrotron based fourier transform infrared microspectroscopy

J. Sulé-Suso[1], Y. Yang[2], M. Tobin[3], F. Bahrami[3], G. Sockalingum[4], A. Kohler[5], M. Manfait[4], A.J. El Haj[2]. [1]*University Hospital of North Staffordshire, Staffordshire Oncology Centre, Stoke on Trent, United Kingdom;* [2]*Keele University, Institute for Science and Technology in Medicine, Stoke on Trent, United Kingdom;* [3]*CCLRC Daresbury Laboratory, Warrington, United Kingdom;* [4]*University of Reims, Unité MéDIAN, CNRS UMR 6142, Reims, France;* [5]*Norwegian Food Research Institute, Ås, Norway*

Background: Chemotherapy has become one of the main treatments for patients with lung cancer. However, it would be ideal to have a tool that could allow clinicians to determine what would be the best combination of chemotherapeutic drugs for each individual patient. To this purpose, we studied whether synchrotron based Fourier Transform Infrared (FTIR) microspectroscopy could become such a tool. This technique uses infrared light that interacts with a sample and measures the vibrational modes of the functional groups of biomolecules present in cells and tissues.
Materials and Methods: Three lung cancer cell lines (A549, CALU-1, and SKMES) were used in this study. Spectra were obtained by seeding 5 x 10^4 lung cancer cells in 100 μL of complete media on aluminium coated slides. After 1 hour incubation at 37ºC and 5% CO_2, gemcitabine at different doses was added to cell cultures. After an overnight incubation, samples were washed three times with 0.9% NaCl. Samples were then kept at −80º before obtaining their synchrotron based micro-FTIR spectra with a Thermo Nicolet Continuum FTIR microscope on beamline 11.1 at the Synchrotron Radiation Source, Daresbury Laboratory, UK. Single cell spectra were recorded with a 10 μm aperture at a resolution of 4 cm^{-1} and with 128 co-additions.
Results: Cell survival decreased proportionally to the addition of gemcitanine. Furthermore, lung cancer cells became rounded and pyknotic following the addition of this drug. This correlated with changes in their micro-FTIR spectra. In fact, the amide I peak at 1645 cm^{-1} (C = O stretching vibrations) shifted to lower wavenumbers following the addition of gemcitabine to *in vitro* growing lung cancer cells. This shift has been associated with cell death.
Conclusion: synchrotron based microscopic FTIR spectroscopy could have a potential as a tool to assess tumour response to chemotherapy at a single cell level.
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OP18/Stathmin expression and phosphorylation influences sarcoma cells invasion and metastasis

M.S. Nicoloso, B. Belletti, S. Berton, M. Schiappacassi, F. Lovat, A. Colombattti, G. Baldassarre. *CRO-National Cancer Institute, Experimental Oncology and Epidemiology, Aviano, Italy*

OP18, also known as stathmin, is a cytosolic phosphoprotein firstly identified to be a relay of several intracellular pathways and to be overexpressed in several types of cancer (thus the name of stathmin or OncoProtein 18). Subsequently, it has been demonstrated that OP18 is a microtubules destabilizing protein whose activity is highly regulated by serine phosphorylation. While the role of OP18 expression and phosphorylation has been largely studied in the regulation of mitotic division whether OP18 influences cancer progression is still unclear. Here we report the role of OP18 expression and phosphorylation in sarcoma cells proliferation and motility, following cell-ECM contacts.
We show that OP18 is phosphorylated on three different serine residues (S17, S25 and S38) following adhesion on ECM substrates principally through the activation of the MAPK pathways. In sarcoma cells OP18 expression enhances their motility but is ineffective on their proliferation rate. A mutant defective for adhesion dependent serine 16 phosphorylation is more able in stimulating cell motility through ECM substrates *in vitro*. Expression of the mutant OP18 protein is also able to increases sarcoma cell spreading potential but not local growth *in vivo* in nude mice. Finally, in a panel of human sarcomas OP18 resulted frequently overexpressed respect to the normal counterpart. Interestingly, Op18 is more expressed in recurrent or metastatic respect to primary samples suggesting that it could play a role in sarcoma local or distant dissemination.

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CARF, a collaborator of ARF, regulates p53 functions by affecting MDM2 expression *in vivo*

K. Hasan[1,2], R. Wadhwa[1], T. Hirano[1], S.C. Kaul[1]. [1]*National Institute of Advanced Industrial Science, Gene Function Research Center, Tsukuba, Ibaraki, Japan;* [2]*Yokogawa Electric Corporation, Tokyo, Japan*

CARF, a collaborator of ARF, was first cloned as a novel ARF-binding protein by a yeast interaction screen. It also interacts with p53 directly leading to ARF-independent enhancement of p53 function and in turn undergoes a negative feed back regulation [1,2]. In the present report we describe the *in vivo* and *in vitro* interactions of CARF with p53-anatagonist, MDM2.
Immunostaining and real time visualization by time-lapse video microscope also showed that these two proteins co-localize in the nucleus. It undergoes proteasome mediated degradation via mdm2 mediated ubiquitination. We also found that siRNA mediated silencing of CARF causes up-regulation of MDM2 and down-regulation of p21 and other p53 targeted genes like BAX and PUMA. However, the level of p53 remains unchanged. Simultaneous knockdown of both CARF and MDM2 in U2OS cells failed to down-regulate the p21 expression.
This finding suggests that accumulation of MDM2 in CARF-depleted cells might be responsible for transcriptional inactivation of p53. The data suggests that CARF regulates p53-p21 pathway by more than one way. In addition to its interactions with ARF and p53 proteins, CARF interacts with MDM2 (a terminator of p53 function) and keeps its level in control.

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Transforming growth factor-beta 1 transiently induces Id-1 mRNA through protein kinase C delta and p38 MAP kinase pathway in MDA-MB-231 cells

E. Yun[1], C. Park[1], K. Song[1], Y. Kim[1], J. Kim[1], K. Seo[1], J. Park[1], J. Park[1], W. Yoon[2,3], K. Lim[1,3,4], B. Hwang[1,3,4]. [1]*College of Medicine, Chungnam National Univ., Biochemistry, Daejeon, Korea;* [2]*College of Medicine, Chungnam National Univ., Surgery, Deajeon, Korea;* [3]*Cancer Research Institute, Daejeon, Korea;* [4]*Institute of Biotechnology, Daejeon, Korea*

Id-1 (Inhibitor of differentiation-1), one of the helix-loop-helix (HLH) proteins, inhibits basic HLH transcription factors from binding to DNA and plays an essential role in the inhibition of differentiation and the cell cycle arrest. To investigate relationship between aggressiveness of breast cancer and Id-1, TGF-β1-dependent regulation of Id-1 has investigated in MDA-MB-231 cells.
The level of Id-1 mRNA is dramatically and transiently increased at 1 hour and disappeared after 3 hours by TGF-β1 in MDA-MB-231 cells (aggressive cells) but was no effect in MCF-7 cells (non-aggressive cells). Id-1 protein was also shown the same result. TGF-β1-induced Id-1 mRNA level was almost reduced by pretreatment of actinomycin-D but enhanced by cycloheximide. In DNase I footprinting analysis, the nuclear factors interacting with the cis-elements were identified in Control and TGF-β1-treated MDA-MB-231: CREB/ATF (−1017) and SBE (−993) binding site, but Egr-1 (−1063) was not detected. The trans-acting factors were bound to each cis-element-specific but quantitative differences were not shown between control and TGF-β1-treated cells. The level of TGF-β1-induced Id-1 mRNA was significantly reduced by Rottlerin (PKCδ-specific inhibitor) and SB 203580 (p38 MAP kinase inhibitor) but was no effect by Gö 6976 (protein kinase Cα, β and μ inhibitor) or PD 98059 (ERK 1/2 inhibitor).
These results suggest that Id-1 may be important to aggressiveness of breast cancer cells and PKCδ and p38 MAP kinase signaling pathway is related to TGF-β1-dependent induction of Id-1 mRNA in MDA-MB-231 cells.