Thymoquinone, is a novel selective microtubule-targeting drug, by inducing the degradation of α and β tubulin in human leukaemia and human astrocytoma cells but not in normal human fibroblasts

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The microtubules-targeting natural substances as such as vinca-alkaloids and taxanes are an important efficient family of anticancer drugs with therapeutic benefits in both blood and solid cancers. These drugs interfere with the assembly of microtubules from α and β tubulin heterodimers without altering their expression levels. Indeed, vinca-alkaloids such as vinblastine and vincistine are known to inhibit tubulin polymerisation into microtubules, while taxanes, such as paclitaxol, have been reported to inhibit microtubule depolymersiation into tubulin[1]. The aim of the present work was to study the effect of thymoquinone (TQ), a natural product with anticancer activities, on α and β tubulin expression in Jurkat cells (T lymphoblastic leukaemia cells) and human astrocytoma cells (cell line U87) as solid tumor. Normal human fibroblasts were used as a non-cancerous cell model. We found that TQ induced a dose- and time- dependent degradation of α and β tubulin in Jurkat and U87 cancer cells. This degradation was associated with the degradation of UHRF1 (Ubiquitin-like containing PHD Ring Finger), an anti-apoptotic protein essential for cell proliferation[2] and the up-regulation of the tumor suppressor p73[3]. We also found that TQ induced caspase-3 up-regulation with subsequent apoptosis. Interestingly, TQ had no effect on α and β tubulin and UHRF1 protein expression in normal human fibroblast cells. These data show that TQ exerts a selective effect towards cancer cells and suggest the existence of a relationship between the down-regulation of α and β tubulin and the down-regulation of UHRF1 in cancer cells.

In conclusion, we propose that TQ is a novel anti-microtubule drug, considering that it targets the levels of α and β tubulin proteins and not their functions. Furthermore, our study highlights the interest of developing anti-cancer therapies that target directly tubulin synthesis.

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Fruit juice-induced endothelium-dependent relaxations in isolated porcine coronary arteries: Evaluation of different fruit juices and purees and optimization of a red fruit juice blend

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Numerous studies have indicated that several polyphenol-rich sources such as red wine and green tea are potent inducers of endothelium-dependent relaxations in isolated arteries. As various fruits and berries are known to contain high levels of polyphenols, the aim of the present study was to assess the ability of selected pure fruit juices and purees as well as blends to cause endothelium-dependent relaxations in isolated arteries. Vascular reactivity was assessed using porcine coronary artery rings, and fruits juices, purees and blends were characterized for their content in vitamin C, total phenolic, sugar and antioxidant activity. Fruit juices and purees caused variable concentration-dependent relaxations, with blackcurrant, aronia, cranberry, blueberry, lingonberry, and grape being the most effective fruits. Several blends of red fruits caused endothelium-dependent relaxations. Relaxations to blend D involved both a NO- and an EDHF-mediated components. The present findings indicate that some berries and blends of red fruit juices are potent inducers of endothelium-dependent relaxations in the porcine coronary artery. This effect involves both endothelium-derived NO and EDHF, and appears to be dependent on their polyphenolic composition rather than on the polyphenolic content.

Aqueous Extract of *Anogeissus leiocarpus* (*AEAL*) induced endothelium-dependent vasorelaxation on porcine coronary artery via a predominent NO-dependent and partially via EDHFs

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Hypertension is a serious public health problem. According to the World Health Organisation (WHO), more than 80% of African population uses medicinal plants to meet its health care requirements. *Anogeissus leiocarpus* possess antifungal, antimicrobial, trypanocidal and antihypertensive properties [1;3]. In Burkina Faso, *Anogeissus leiocarpus* is used in traditional medicine to treat several pathologies such as Arterial Hypertension (HTA) [2]. Our objective was to characterized endothelium-dependent relaxations to *AEAL*, extracted from *Anogeissus leiocarpus* bark of trunk, on porcine coronary artery and its effects on phosphodiesterases (PDEs) inhibition.

The results show concentration-dependent and *in intro* significant inhibition of PDEs (1-5) with better activity on the PDE1, activated by calmoduline (IC_{50} =7.5 ± 4µg/mL). The IC_{50} are of 21.4 ± 5.7: 83.7 ± 17.2; 60.6 ± 13.8 and 41.8 ± 17.3 for PDE2, PDE3; PDE4 and PDE5 respectively.
On porcine coronary isolated arteries rings pre-contracted with U46619, the relaxations effects to *AEAL* are endothelium-dependent. Indeed, endothelium-dependent relaxations to *AEAL* were abolished in endothelium-denuded coronary artery rings. The same effect was obtained in the presence of N^{ω}-nitro-L-arginine (LNA, 300µM, an inhibitor of endothelial NO synthase), by the combination of L-NA plus charybdotoxin (CTX, 100nM) plus apamin (APA, 100nM) (inhibitors of EDHF-mediated responses) and in the presence of MnTMPyP (100µM, inhibitor of ROS).
The endothelium-dependent vasorelaxation to *AEAL* is slightly but significantly reduced by the combinaison of CTX plus APA, in the presence of PP2 (10µM, Src kinase inhibitor) and wortmannin (30nM, PI3-kinase inhibitor). In cultured endothelial cells, *AEAL* induce ROS generation but not in the combination with MnTMPyP.

Taken together, *AEAL* induced endothelium-dependent relaxations on porcine coronary arteries, which involve a NO-mediated component but also, partially, an EDHF-mediated component.

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Blocking of SIRPα-CD47 interaction promotes the proliferative effect of mast cells on human astrocytoma cell lines via ERK and Akt phosphorylation

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Signal regulatory protein α (SIRPα) is a transmembrane glycoprotein receptor expressed mainly in myeloid and neuronal cells. The extracellular region of the molecule is composed of immunoglobulin (Ig)-like domains that mediate recognition of the broadly expressed cellular ligand CD47. Interaction between CD47 and SIRPα constitutes a cell-cell communication system that plays important roles in a variety of cellular processes, including cell proliferation [1]. Our objective was to study the proliferative effect of rat peritoneal mast cells (RPMC) on human astrocytoma cell lines U87, CCFSTTG1, U373 and SW-1783 by blocking the SIRPα-CD47 interaction using monoclonal antibody raised against recombinant extracellular domain of human SIRPα (SE7C2).

Human astrocytomas cells lines CCF-STTG1 and U87 (WHO grade IV), U373 and SW-1783 (WHO grade III) were co-cultured with RPMC (ratio 1:4 and 1:2) and incubated with 1.5 and 2.5 µg/ml of SE7C2. Proliferation was assessed by the colorimetric MTS test and FACS assay. SIRPα, CD47 receptor expression were evaluated by RT-PCR and ERK, Akt, p70s6k phosphorylation by western blot.

The SIRPα and CD47 receptor expression by human astrocytoma cell lines was confirmed by RT-PCR and western blot. The blocking of SIRPα/CD47 interaction by SE7C2 promotes the proliferative effect of RPMC on human astrocytoma cell lines U87 and CCF-STTG1 but not on U373 and SW-1783. Furthermore, RPMC decrease CD47receptor and SIRPα expression on U87 cell line, which suggests that cells induce the proliferation of astrocytoma cell lines by down regulation of these proteins.
Other results showed that SE7C2 induces alone the proliferation of U87 and CCF-STTG1 cell lines and increases ERK, p70s6k phosphorylation by U87 and Akt, p70s6k phosphorylation by CCF-STTG1.

Our data indicate that SIRPα monoclonal antibody SE7C2 promotes the growth of human astrocytomas cells lines co-cultured with RPMC via inhibition of the negative signal induced by SIRPα, which promotes the phosphorylation of proteins involved in cell survival, thereby likely contributing to tumor aggressiveness by enhancing the proliferation.

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Phytochemical study and evaluation of anti-inflammatory activity of *Dodonaea viscosa*

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Dodonaea viscosa (L.) Jacq. (Sapindaceae) is a medicinal plant growing in the tropical regions and frequently used for example in Cameroun. Ethyl acetate extract from the leaves was subjected to column chromatography on silica gel.

Seven compounds were isolated [1] : a new labdane diterpenoid 2,19-dihydroxylabda-7,13(*E*)-dien-15-oic acid (**1**) and a new 3-methoxyflavone, 5,7-dihydroxy-3,6,4'-trimethoxy-3'-(4-hydroxy-3-methyl-but-2-enyl)flavone (**2**) together with five known compounds including two labdane-type diterpenes, 2,17-dihydroxylabda-7,13(*E*)-dien-15-oic acid (**3**) and 2-hydroxylabda-7,13(*E*)-dien-15-oic acid (**4**), and three 3-methoxyflavone derivatives, 3,6-dimethoxy-5,7,4'-trihydroxyflavone (**5**), 5,4'-dihydroxy-3,6,7-trimethoxyflavone or penduletin (**6**) and 5,7-dihydroxy-3,6,4'-trimethoxyflavone or santin (**7**). Their structures were established by extensive analysis of spectroscopic data (1D and 2D NMR, MS).

The anti-inflammatory activity [2] of five compounds (**1 – 5**) was evaluated with a flow cytometry TNF-α secretion assay on human THP-1 cell line. Compound (**3**) exhibited the highest anti-inflammatory activity with 46% of inhibition.

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Evaluation of the anti-cancer properties of different berry juices in several colon cancer cell lines : Characterization of the underlying mechanism

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Polyphenols are natural compounds widely present in fruits and vegetables, which have strong antimutagenic and anticancer properties. In the present study, several polyphenol-rich berry juices containing about 5 g/L total polyphenols assessed as gallic acid equivalents were screened using the MTS cytotoxicity test for their antiproliferative effect in human colon cancer cell lines: HCT116, HT29, Caco-2, and SW480. Chokeberry (*Aronia Melanocarpa)*, black-currant *(Ribes nigrum)*, and cranberry *(Vaccinium macrocarpon)* juices inhibited markedly HCT116 cell proliferation whereas blueberry *(Vaccinium Cyanococcus)* and lingonberry (*Vaccinium vitis-idaea)* juices were less effective. The most effective juice was cranberry juice with an IC_{50} values in the range of 75-100 µg/ml. These juices were also effective, although to a lesser extent, against other human colon cancer cell lines including HT29, Caco-2, and SW480. The cell cycle was assessed by flow cytometry, and the expression of target proteins by Western blot analysis. The antiproliferative effect of the cranberry juice in HCT116 was associated with cell cycle arrest in G2/M phase in a concentration-dependent manner. These effects were also associated with an upregulation of the tumor suppressor p73, and a downregulation of cyclin B1 and the epigenetic integrator UHRF1. Altogether, these findings indicate that several polyphenol-rich berry juices are able to inhibit the proliferation of human colon cancer cell lines and, hence, they might have a potential in the treatment of human colon cancer.

Activity of soluble CXCL12 neutraligands *in vivo* in a short model of asthma

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The CXCL12 chemokine plays an important role in inflammation. Our team identified a CXCL12 neutraligand (C05) belonging to the family of chalcones, that inhibits its interaction with CXCR4 and CXCR7 (*JBC, 2008*) and the eosinophil infiltration in a model of asthma. Due to its low solubility (C05, 9µM), we decided to facilitate its administration by adding three types of groups: phosphate (C05-P), L-serine (C05-Ser) and sulfate (C05-S), as prodrugs.

The analytical characterization of the three compounds was performed on the Techmed^{ILL} platform (www.pcbis.fr): solubility and stability ($t_{1/2}$) were measured in serum, PBS and lung homogenate. The *in vitro* activity was evaluated by intracellular calcium assay and *in vivo* in a 8-day asthma model: Balb/c mice were sensitized (OVA+alum, i.p, D0,1,2) and challenged to OVA or saline (i.n, D5,6,7). Drugs or solvent were administered i.p or i.n 2h before each challenge.

Prodrugs are 5-10000 times more soluble than the chalcone C05. None of these prodrugs modifies the calcium impulse induced by CXCL12. On the other hand, after incubation in mouse serum or lung homogenate, the prodrugs are transformed into C05 (HPLC). *In vivo*, at 350µmol/kg i.p, C05-P (PBS) has the same activity as C05 (CMC 1%) with 80% inhibition of eosinophil recruitment in BAL. C05-P vs C05 administered i.n at a dose of 22µmol/kg (limit of C05 solubility in PBS) inhibits the eosinophil recruitment by 50% *vs* 0%, respectively. C05-P i.n shows an activity 10000 times higher (IC50=10±7nmol/kg) than i.p administration. A comparison between compounds C05-P, C05-Ser and C05-S (i.n) at 30nmol/kg shows similar activity (67, 69 and 64% respectively).

Use of soluble CXCL12 neutraligand prodrugs enable local administration and activity at low doses and offers an interesting strategy for the development of drug candidate for asthma treatment. Furthermore, our short model of asthma is highly efficient to estimate new therapeutic strategies.

Single-chain Fv fragment antibodies selected from an intrabody library as effective mono- or bivalent reagents for in vitro protein detection.

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In spite of their many potential applications, recombinant antibody molecules selected by phage display are rarely available commercially, one reason being the absence of robust bacterial expression systems that yield sufficient quantities of reagents for routine applications. We previously described the construction and validation of an intrabody library that allows the selection of single-chain Fv (scFv) fragments solubly expressed in the cytoplasm. Here, we show that it is possible to obtain monomeric scFvs binding specifically to human papillomavirus (HPV) type 16 E6 and cellular gankyrin oncoproteins in quantities higher than 0.5 g/L in E. coli cytoplasm after auto-induction. In addition, stable bivalent scFvs of increased avidity were produced by tagging the scFvs with the dimeric glutathione-S-transferase enzyme (GST). These minibody-like molecules were further engineered by fusion with green fluorescent protein (GFPuv), leading to high yield of functional bivalent fluorescent antibody fragments. Our results demonstrate that scFvs selected from an intrabody library can be engineered into cost-effective bivalent reagents suitable for many biomedical and industrial applications.

Synthesis and characterization of fluorophores with heart diceto-pyrrolo-pyrrole (DPP) for the two photon microscopy

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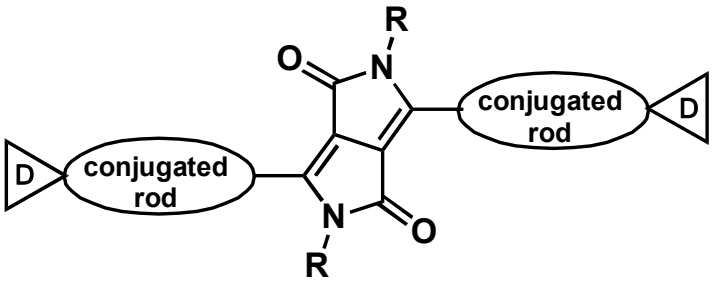
Two-photon induced fluorescence is nowadays widely used for the imaging of biological tissues. Instead of absorbing a single photon of energy *h*v to reach an excited state, a fluorophore can absorb two photons of energy *h*v/2. This involves the use of an infrared light which is less energetic than the UV-visible lights classically used in confocal microscopy. Furthermore it allows to reduce the photodamage of biological tissues and to observe in depth even in diffusing media. The precise spatial localization of the two-photon excitation phenomena allows also to limit the photobleaching in the whole sample. The classical fluophores used in confocal microscopy exhibit low sensitivity to two-photon excitation for the two-photon excitation microscopy (TPEM), led the researchers towards the development of new fluorophores, specifically engineered for TPEM.

We will present our work on the synthesis of new one-dimensional fluorophores containing based on dicéto-pyrrolo-pyrrole (DPP) central core, surrounded by various electro-active systems. By specific molecular engineering we so elaborated new fluorescent dyes with a red-shift fluorescence and with high solubility s in the water to facilitate their use in biological experiments.

The photophysical properties of these systems will be reported as well as the preliminary TPEM images for HeLa cells stained with these new dyes. We highlight a fast penetration of fluorophores in the cytoplasm (< 2 mn), but no nuclear penetration. In conclusion our studies open new perspectives in biological imaging by two-photon microscopy with bioconjugatable dyes.



TechMed^{ILL} - Drug technologies platform

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Search of new potentially therapeutic agents is now easier thanks to recent advancements in genomics, proteomics, medicinal chemistry and especially high-throughput screening methodologies. From the large number of hits generated by these technologies, researchers need to select and optimise the most promising compounds.

One of the major step of this drug discovery process is the study of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity). The determination of these properties at an early stage of development is essential to save time and money.

TechMed^{ILL} is a technologic platform, located in ESBS, which provides services in the area of ADMET. The platform is established since 2008 and works with academic laboratories and biotech companies. The portfolio of assays includes all aspects of the ADMET properties of small drug molecules.

Quality management of the TechMed^{ILL} platform has been recognized by the international ISO 9001 certification.

Investigation by atomic force microscopy of the interaction of a HIV-1 protein with model membranes

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The human immunodeficiency virus type 1 (HIV-1) is a retrovirus of the lentivirus family responsible of the acquired immunodeficiency syndrome (AIDS) which kills almost 2 millions of people all over the world per year. Nowadays, treatments are available that can keep patients alive with acceptable life conditions, but there is still no medicine to cure AIDS.

For productive cell infection, the HIV-1 genome requires to be reverse-transcribed into double stranded DNA, which will then be integrated into the host genome to produce the viral proteins. Subsequently, the produced proteins and notably the Gag polyprotein will recruit the genomic RNA at the plasma membrane, in order to produce a new viral particle that will bud and mature. These various steps of the HIV-1 replications cycle, require the cooperation of both viral and host proteins. One key viral protein playing numerous roles in the viral replication is the nucleocapsid protein (NCp7), a 55 amino-acid protein resulting from the cleavage by the HIV-1 protease of the Gag polyprotein Gag. Each virus contains around 1500 NCp7 molecules bound to the dimeric genomic RNA. NCp7 is characterized by two zinc atoms bound to CCHC zinc fingers responsible together with the numerous basic residues of the nucleic acid chaperone properties of NCp7. These chaperone properties play an important role in the reverse transcription step and, the integration of the viral genome into the host genome.

NCp7 within the Gag polyprotein is also required to specifically recognize the genomic RNA within the large excess of cellular RNAs. As a part of Gag or as a free protein, NCp7 may also bind to membranes. This possibility has been studied with atomic force microscopy (AFM), an imaging technique that provides 3D pictures of different surfaces with both high lateral and axial resolutions (the order of the nanometer). Experiments were performed with the intermittent contact AFM mode in liquid phase. We used supported lipid bilayers (SLBs), composed of a mixture of two lipids, on mica, as model membrane. These AFM investigations show that NCp7 interacts efficiently with model membranes. These data correlate well with dynamic light scattering (DLS) and fluorescence spectroscopy data, suggesting that membranes may constitute additional partners of this protein.

Intake of grape-derived polyphenols prevents doxorubicin-induced endothelial dysfunction in the rat mesenteric artery

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Doxorubicin is a potent chemotherapeutic agent effective against many types of cancers. Unfortunately, its clinical use is restricted by its cardiotoxicity, which involves free radical formation. This study aims to determine whether doxorubicin affects vascular reactivity and, if so, to assess the preventive effect of red wine polyphenols (RWPs), a natural source of antioxidants. Male Wistar rats (12 weeks) were assigned to 4 groups; control, doxorubicin (15 mg/kg, i.p), RWPs (75 mg/kg/d in the drinking water for 22 weeks) and doxorubicin + RWPs. The reactivity of mesenteric artery rings was assessed in organ chambers, the vascular formation of reactive oxygen species (ROS) using dihydroethidine and the expression level of small and intermediate conductance calcium-activated potassium channels (SK_{Ca}, IK_{Ca}), connexin 40 (Cx40), endothelial NO synthase (eNOS), angiotensin II and AT1 receptors in mesenteric artery segments by immunohistochemistry. The EDHF-mediated relaxations to acetycholine were blunted in mesenteric artery rings of doxorubicin-treated rats in comparison to those of control rats whereas the NO-mediated relaxations were not affected. Impaired EDHF-mediated relaxations were associated with reduced expression of SK_{Ca}, IK_{Ca} and Cx40 in the arterial wall. Doxorubicin treatment increased the vascular formation of ROS and the expression of eNOS, angiotensin II and AT1 receptors. Intake of RWPs prevented the effects of doxorubicin on vascular reactivity, formation of ROS and expression of target proteins. Chronic treatment of rats with doxorubicin induced blunted EDHF-mediated relaxations which is due, at least in part, to a decreased expression of SK_{Ca}, IK_{Ca}, and Cx40 in the arterial wall. This is presumably the result of enhanced angiotensin II formation, which activates AT1 receptors leading to vascular oxidative stress. Intake of RWPs is able to prevent the doxorubicin-induced endothelial dysfunction most likely by preventing oxidative stress.

Thymoquinone reduces U-87 cells invasion through FAK down-regulation

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Glioblastoma represent the most frequent primary tumors of the central nervous system and remain among the most aggressive human cancers as available therapeutic approaches still fail to contain their invasiveness. Many studies have reported elevated expression of the Focal Adhesion Kinase (FAK) protein in glioblastoma associated with an increase in the rates of invasion [1,2]. This designates FAK as a promising target to limit invasiveness in glioblastoma. Thymoquinone (TQ), the main phytoactive compound of *Nigella sativa* has shown remarkable anti-neoplasic activities on a variety of cancer cells, mainly through its anti-oxydant, anti-proliferative, anti-inflammatory and immuno-modulatory effects [3]. Few studies have also described anti-invasive and/or anti-migratory properties of TQ on colorectal [4], lung cancer models [5], and human umbilical vein endothelial cells [6], but the underlying molecular mechanism remains poorly understood Here, we studied the effects of TQ on human U-87 glioblastoma cells invasion.

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Towards new optogenetic tools for investigating P2X receptors

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P2X receptors are trimeric ligand-gated ion channels that are selective to cations. These ion channels (7 homologous so far identified in mammals), activated by extracellular ATP, are involved in various physiological processes such as synaptic transmission, inflammation and pain sensation and therefore they represent promising targets for new therapeutic agents. Very recently, we have identified the ATP-binding site in the P2X2 receptor and localized its position in the recent X-ray structure of the zebrafish P2X4 receptor solved in the absence of ATP. Our results thus provide new structural insights into the ATP binding site; however, the mechanism by which ATP-binding couples to channel opening remains unknown.

Our study aims to develop new ATP photoisomerizable analogues able to photoregulate the P2X activity. This optogenetic strategy consists in covalently tethering the ATP analogue to a cysteine, properly introduced near the ATP binding site. Depending on the wavelength of photo-stimulation, the ligand reaches (or not) its ATP binding site and activates (or not) the receptor. This optical switch allows a spatiotemporal control of the targeted protein and offers interesting outlooks on the structure-function relationships.

Molecular characterization of a putative new effector of G-protein coupled receptors

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We have performed a functional live-imaging screen of a full-length human cDNA library that comprises GFP-tagged Open-Reading-Frames, concentrating on those fluorescent clones whose expression in the cell cytoplasm was affected by activation of G-protein coupled receptors (GPCR). This particular screening procedure was chosen to identify unknown cytoplasmic proteins implicated in the signalling pathways of GPCRs using the tachykinin NK2 receptor as a model. We found the 1622 clone localized to the cytoplasm and nucleus but following activation of the NK2 receptor, this fluorescent protein translocated transiently to the plasma membrane.

The human 1622 protein is a member of a gene family that includes one homolog in human and two orthologs in *Drosophila*. These proteins exhibit no homology with other known proteins nor do they contain any recognizable functional domains. There are no reports in the literature for a role of these proteins at the plasma membrane or in the GPCR signaling pathways. However, these proteins have been reported to inhibit growth factor signalling that leads to activation of the mammalian Target of Rapamycin Complex 1. This is via a mechanism that has not been characterized at the molecular level but that implies activation of the Tuberous-Sclerosis-Complex 1/2 down-stream of AKT.

We have quantified plasma membrane localization in living cells, using a Bioluminescence Resonance Energy Transfer (BRET) assay. Surprisingly, this shows that the 1622 protein, is already largely associated with the plasma membrane at steady state, giving a BRET signal as high as several control integral plasma membrane proteins. Furthermore, the BRET signal is increased following activation of several endogenously expressed receptors such as beta-2-adrenergic, muscarinic m3 or adenosine-b2 receptors, but not upon growth factor receptor stimulation. These two biochemical properties are not shared with its homolog. We are now investigating which unique domains of this protein, absent in its homolog, are involved in i) the localization at the plasma membrane and ii) its translocation upon agonist stimulation of cells, and what are the mechanisms accounting for membrane localization.

At the same time, we are investigating the functional effects of the modulation of the 1622 protein and several mutants on the GPCR signaling cascades, in particular on mTORC1 modulation of activity by GPCRs.

The final aim of this project is to define the place of the 1622 protein as an effector in GPCR signaling cascades.

The proteome of DNA repair foci

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B lymphocytes are unique in that they diversify their antigen receptor genes through somatic hypermutation (SHM)[1] that modify the affinity of the B cell receptor for antigen by introducing point mutations in the immunoglobulin (Ig) variable region, while Class Switch Recombination (CSR) modulates antibody effector functions by replacing the antibody isotype expressed[2]. These reactions are initiated by DNA damage inflicted specifically at immunoglobulin (Ig) genes by Activation Induced Cytidine Deaminase (AID)[2] an enzyme that deaminates cytosine residues in DNA[3]. During CSR, AID-induced DNA damage generates double stranded DNA breaks (DSBs), which are obligate intermediates in the reaction[2]. AID-induced lesions are recognized by components of the DDR and later repaired by non-homologous end joining[4].

The DNA damage response (DDR) manifests itself morphologically in the form of DNA repair foci, structures formed by the recruitment and accumulation of DNA repair factors at a site of DNA damage[1]. As a consequence, a single double stranded break (DSB) nucleates a macroscopically discernible focus, which spreads up to one megabase in the surrounding chromatin, and is believed to be essential for promoting efficient DNA repair[2].

We propose to determine and compare the identity of proteins that accumulate into nuclear foci at a particular genomic locus in response to a restriction enzyme and AID-induced breaks and functionally characterize the novel factors identified.

We generated stable cell lines bearing multiple tandem integrations of a cassette containing the 18 nucleotide I-SceI restriction site followed by an array of 256 copies of the lac-repressor binding site (lac operator: lacO). This array allows us to generate DSBs in a single locus by expressing the I-SceI homing endonuclease and chimeric AID proteins fused to the LacI repressor (LacI) that targets AID to the lacO chromatin, as it has been done with other proteins[5]. To identify proteins that accumulate into nuclear foci in response to DSBs induced by AID and I-SceI we will use the technique of proteomics of isolated chromatin segments (PICh)[6]. This method is based on the formaldehyde crosslinking of interacting proteins bound to DNA followed by the precipitation of specific genomic loci using desthiobiotin-tagged oligonucleotides containing locked nucleic acid (LNA). The DNA/probe hybrids are isolated using magnetically-labeled avidin beads. The crosslink is then reversed and co-precipitating proteins are resolved by SDS-PAGE and identified by mass spectrometry.

By this approach we expect to find proteins that play different roles in DNA repair processes: DNA damage sensors, signaling/transducer proteins of the DNA damage response, chromatin modifiers and proteins that facilitate the actual ligation of the broken ends.

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MSK1 kinase overexpression is related to the inflammatory and fibroproliferative infiltration in a mouse model of obliterative bronchiolitis

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Introduction : Obliterative bronchiolitis (OB) is the manifestation of chronic allograft rejection after lung transplantation and is characterized by obstruction of the small airways with inflammatory infiltrate and fibrosis. Our hypothesis proposes the nuclear kinase MSK1 (Mitogen- and Stress-Activated Kinase) as an actor in OB *via* the activation of pro-inflammatory genes.

Methods : A mouse model of heterotopic tracheal transplantation (iso- and allograft) was used. Mice were treated intraperitoneally up to 21 days (D) with compounds inhibiting MSK1: H89 (10 mg/kg/day) and fasudil (30 mg/kg/day) *vs* solvent (DMSO 5%). Total RNA was extracted from tracheas and MSK1 and IL-6 mRNA levels were quantified by qPCR. Tracheal sections were observed after hematoxylin-eosin staining. CD3+ lymphocyte infiltration was quantified at D7 after immunohistochemistry.

Results : MSK1 mRNA is increased in the allografts by +68±8% at D7 and +85±4% at D21 in comparison to D0, whereas the expression of MSK1 mRNA is unmodified in the isografts. Likewise, the expression of IL-6 mRNA in the allografts at D7 is increased by +88±2% as compared to D0. Histologically, the allograft shows inflammatory infiltration at D7 and lumen fibroproliferative obstruction at D21. Upon H89 and fasudil treatment, a decreased lymphocyte infiltration is observed, of 92±3% for H89 and 90±5% for fasudil at D7 as compared to controls. At D21, H89 and fasudil inhibit the tracheal obstruction in allografts by 80% and 45%, respectively, compared to controls. No effect of H89 or fasudil is observed in isografts.

Conclusion : Our data show that the nuclear kinase MSK1 is overexpressed upon graft rejection in our model of OB, as well as the pro-inflammatory cytokine IL-6. Pharmacological inhibition of MSK1 causes a reduced lymphocytic infiltration and tracheal obstruction. Thus, MSK1 is proposed as a potential therapeutic target to combat OB after lung transplantation.

Radical scavenging effects of fruit extracts from two *Ficus* species

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Ficus gnaphalocarpa L. and *Ficus dekdekena (Miq) A.Rich.* belong to the well-known plants of Moraceae family wich include many species growing in tropical regions. The fruits of these two Ficus species are traditionally used as dietary wild fruits in West africa [1].

The aim of our study was to evaluate the *in vitro* radical scavenging activities (RSA) of *F. dekdekena and F. gnaphalocarpa* fruits hydroalcoholic extracts, using two different free radicals: hydroxyl radical ($HO^{\cdot}$) and superoxide anion ($O_2^{\cdot -}$).

At doses of 100 and 25 µg.mL^{-1} the percentage of inhibition of $O_2^{\cdot -}$ values obtained for extracts were 61,41 and 24,02 for *F. dekdekena*, 23,3 and 31,77 for *F. gnaphalocarpa respectively*.

At 10 and 2 µg.mL^{-1} the percentage of inhibition of $OH^{\cdot}$ values obtained for extracts were 88,37 and 52,51 for *F. dekdekena*, 87,09 and 50,28 for *F. gnaphalocarpa respectively*

Preliminary phytochemical investigation of the extracts using HPLC-DAD and LC-MS showed the presence of protocatechuic acid, chlorogenic acid, ferulic acid, homo-orientin, rutin, hyperoside and catechin derivatives well known as radical scavenging compounds [2, 3].

These results suggest that *F. dekdekena* and *F. gnaphalocarpa* fruit extracts possess radical scavenging activity, wich could be attributed to the presence of cinnamic acid derivatives, catechin derivatives and flavonoids.

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Natural product Marie-Lise database development for high-throughput phytochemical profiling of plant extracts

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Modern drug discovery is greatly based on the identification and structural characterization of new lead compounds, stemming from the huge diversity of natural plant chemicals. The process is tedious facing the complexity of plant metabolome, the high number of samples studied, and the time-consuming steps of purification. In order to reduce the number of *de novo* purification and elucidations of chemical entities, an interesting strategy is to create a natural-product database. We created such a database, named Marie-Lise, using commercial standards and purified molecules from our in-house chemical library (UMR 7200). Alkaloids, steroids, terpenes and phenolic compounds of diverse kinds composed the Marie-Lise database. Analytically, we combine Galaxie and MassLynx software programs and high detection sensitivity and selectivity methods (e.g. HPLC-DAD and LC-QqTOF-MS/MS) in order to create the database and then to discriminate common metabolites from potential lead compounds. This approach allowed us to characterize two hundred pure substances. We also succeeded in associating quantitative data to qualitative value for some compounds by obtaining metabolite specific calibration curves. Thus, building a database of purified natural products allows us to achieve high-throughput screening (HTS) of chemical structures and to accelerate the discovery of new compounds or to identify known compounds of specific biological interest or toxicity, from complex mixtures such as plant extracts or preparations.

Analysis of the Pro-apoptotic activity of natural molecules by capillary cytometry

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The chronic inflammatory bowel disease (CIBD), just like hemorrhagic ulcerative colitis or Chron's disease is primarily due to a deterioration in the intestinal mucosal membrane following a dysregulation in the immune system. In certain cases this disease may lead to colocteral cancer (1 to 2%). The beneficiary effects exhibited in patients treated with non-steroidal anti-inflammatory molecules (NSAI) seem to indicate an apoptotic deficiency at the level of the digestive epithelium.

Recent technological advances such as screening platforms and diversified libraries have greatly facilitated the development of several screening tests, specifically those related to the identification of cellular pro-apoptotic activity. We have previously screened a library containing approximately 7000 molecules from which we selected the molecules that demonstrated the strongest apoptotic potential. We have also used the same screening test in order to evaluate the toxic effects of pure as well as diluted essential oils using monocytic and colonic human cell lineages; results revealed that these oils are comprised of several bioactive molecules possessing pharmacological potentials that can be possibly implicated in the treatment of chronic inflammatory bowel diseases and associated cancers.

The iron acquisition systems in *Pseudomonas aeruginosa* : a target for new antibiotics

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The aim of this project is to develop innovative antibiotic strategies to inhibit *P. aeruginosa's* growth. This pathogenic Gram-negative bacterium is responsible for severe infections affecting particularly cystic fibrosis and immuno-compromised patients. In this context, we wish to validate bacterial iron acquisition systems as biological targets for a new generation of antibiotics. Despite its very low bioavailability, iron(III) is an essential nutrient for the growth and the virulence of *P. aeruginosa*. In order to acquire iron(III) from a host or from the environment, bacteria excrete low molecular weight chelating molecules, called siderophores, to solubilize iron (III) in the extracellular medium. The ferric siderophores are then recognized and assimilated by a transmembrane multiproteic system that requires the energy provided by the TonB machinery. A siderophore called pyochelin is common to all the *P. aeruginosa* strains (including clinically isolated ones) and is therefore a good candidate for the development of Trojan horse antibiotics strategies. In this context, an antibiotic vectorized by pyochelin could be efficiently and selectively transported in the bacteria using the pyochelin-dependent iron uptake pathway. Thus, we synthesized a N3"-functionalized pyochelin which can be used as a vector in such approach. Three different antibiotics from the fluoroquinolone family were connected to the functionalized pyochelin, using either a stable spacer arm or a hydrolyzable one in vivo. The resulting conjugates where then tested on different *P. aeruginosa* strains. Unfortunately, they did not show a higher activity than the non-vectorized antibiotics. We demonstrated that this result was mainly due to the weak solubility of our conjuguates and to an extracellular hydrolysis of the spacer arms. Nevertheless, using fluorescently labelled N3"-functionnalized pyochelin in fluorescent microscopy, we proved that the N3"-functionnalized pyochelin is able to vectorize xeniobiotics into the bacterial cell. We thus pursued the development of new pyochelin-antibiotic conjugates with, at one and at the same time, more resistant spacer arms and an increased solubility in physiological media. In parallel, we also try to use this functionalized pyochelin to vectorize innovative antibiotics currently in development in our laboratory (e.g. Inhibitors of the TonB machinery).

α5β1 integrin antagonists inhibit human glioblastoma tumorigenicity

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Integrins are αβ heterodimeric transmembrane proteins involved in cell adhesion, migration and cell survival often affected in cancer. The α5β1 integrin is over-expressed in glioblastoma, one of the most aggressive brain cancers. Only a few antagonists of this protein are known, a monoclonal antibody (Volociximab) and a polypeptide (ATN-161) are currently tested in clinical trials. Recently a new class of small non-peptidic antagonist has been described. We used one of these molecules (K34c), to study their impact on α5β1 integrin signaling pathways and functional effects in human glioblastoma cell lines.

U87MG and U373 cell lines expressing different levels of the α5 integrin subunit (by genetic manipulations) were treated with K34c. Total and phosphorylated AKT, total and phosphorylated FAK (focal adhesion kinase) were assessed in the absence or presence of K34C by western blotting. Cell clonogenic potential were quantified as a measure of cell survival. Cell migration was quantified by a wound-healing assay.

The inhibition of α5β1 integrin with K34c dose dependently decreases Akt activation in U87 cell lines. The effect on AKT pathway was related to the level of α5 integrin expression. Clonogenic assays showed that K34c was able to decrease the U87MG cell survival suggesting that inhibition of AKT pathway is related to cell survival. Inversely, U373 cells treated with K34c did not show any effect on AKT activation. The FAK phosphorylation on tyrosine 397 was not affected in U87MG cell treated with K34c. Inversely, FAK phosphorylation was decreased in U373 cells. FAK activation is known to be implicated in cell migration. We therefore tested the capability of cells to migrate in the absence or presence of the integrin antagonist. In wound healing assays, we showed that migration was inhibited equally in the U87MG cells expressing high and low level of α5 integrin. Conversely, in U373 cells, migration was inhibited only in cells expressing the α5 subunit. These results suggest that the α5β1 integrin antagonist K34c may have cell specific effects affecting either survival and/or migration depending on the cell characteristics.

Our results highlight the α5β1 integrin as a potential therapeutic target in glioblastoma. They underline the need to develop and characterize new antagonists which will be more selective and more efficient to inhibit the pro-tumorigenic functions of α5β1 integrin in human glioblastoma.

Tumor necrosis factor receptor-associated factor 4 (TRAF4), a new phosphoinositide binding protein

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TRAF4 is a ubiquitous gene that is overexpressed in around 40% of carcinomas. This gene encodes an adaptator protein that belongs to the TRAF family. TRAF proteins are cytoplasmic mediators involved in the initial steps of intracellular transduction induced by several cytokines like the Tumor Necrosis Factor α (TNF) and the Interleukine-1. TRAF4 is a unique member of this family, first through its involvement in cancers and second by its absence of function in immunity. It has been shown in polarized epithelial cells that TRAF4 is associated with the plasma membrane in tight junctions. Up to now, the molecular mechanism leading to TRAF4 membrane recruitment remains unknown. Indeed, TRAF4 does not have any plasma membrane targeting motif and none of its known partners localize in the tight junctions.

Several proteins are recruited to membranes through a lipid binding domain. For example, it has been shown that PAR-3 (Partitioning defective 3) is recruited to tight junctions by interacting with phosphoinositides (PI) via its second PDZ domain. We have hypothesized that TRAF4 was recruited to tight junctions thanks to a lipid binding motif. This hypothesis was tested *in vitro* using recombinant TRAF4 proteins on membranes coated with different lipids. The results show that TRAF4 interacts with phosphoinositides and phosphatidic acid through its TRAF domain. Structural analyses of several PI binding domains have shown that basic residues (lysine and arginine) directly interact with phosphoinositide phosphate. So, we are performing several TRAF mutants where conserved basic residues are mutated to know the potential requirement of PI binding for TRAF4 recruitment in the tight junctions.

Tight junctions are needed for epithelial cells polarity establishment and loss of this polarity is a key process during cancer progression and spreading. A better understanding of TRAF4 recruitment mechanism and function in tight junctions could help to understand the pathophysiological role of TRAF4.

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Structural and functionnal studies of the HIV-1 preintegration complex nuclear import

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Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein (IN) which is a key protein for HIV infection. It is present in the preintegration complex (PIC). PIC is a large nucleoprotein complex of variable component and size which is formed after reverse transcription. The complex is transported to the nucleus through nuclear pore. Integrase is required for reverse transcription, PIC migration along microtubules, transfer to the nucleus, chromatin targeting and integration.

At present, PIC's spatial organization and way of functioning are not well known. The main objective of Marc Ruff's group is to characterize temporally and structurally PIC's complex. A cryo-EM structure at 14 Å resolution of the HIV-1 integrase in complex with the lens epithelium-derived growth factor (LEDGF) has been solved in presence and absence of DNA (1). Recently we solve a cryo-EM structure of the ternary complex of HIV-1 Integrase, LEDGF and the integrase binding domain of the integrase interactor 1 protein (INI1) in absence (20 Å resolution) and presence (16 Å resolution) of DNA.

In this context, I am focusing on the complex with Transportin-SR2 involved in the PIC nucleus import. We characterized one stable complex: IN / VBP1 / Transportin-SR2. Preliminary data on these complexes will be presented. The preparation of the different construct, the optimization of the production of the individual proteins, as well as , preliminary data on in vitro complex reconstitution will be presented.

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Tubulin modification: validation as a therapeutic target for prostate cancer progression

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Prostate cancer is a common cause of death, and an important goal is to establish the pathways and functions of causative genes. We isolated RNAs that are differentially expressed in macrodissected prostate cancer samples. This study focused on 1 identified gene, TTLL12, which was predicted to modify tubulins, an established target for tumour therapy. TTLL12 is the most poorly characterized member of a recently discovered 14-member of the TTLL superfamily, proteins that catalyze posttranslational modification of tubulins.

Tubulin tyrosine ligase (TTL), the founding member of the family, readds tyrosine to α-tubulin that has been terminally detyrosinated producing a ''TTL cycle.'' The TTL cycle is important for neuronal organization, trafficking of intermediate filament proteins and cell morphology and spindle positioning. TTL expression is suppressed during tumor progression, and the resulting increase in detyrosinated tubulin is associated with increased tumor aggressiveness. TTLL1, 4, 5, 6, 7, 9, 11 and 13 have been shown to polyglutamylate a-tubulin and/or b-tubulin and other substrates. TTLL2 is predicted to have polyglutamylase activity. TTLL3 and TTLL10 are tubulin glycine ligases. TTLL12 is the only family member without an assigned enzymatic function.

Tubulins are an important target for tumor therapy, and many compounds are still being developed. They are classified according to their effects on tubulin dynamics (polymerization and depolymerization) and their binding sites. However, their precise mechanisms of action are still poorly understood, and more precise tubulin-targeted molecular therapeutics could be useful. Posttranslational modifications are a good target for the development of new therapeutic agents, as has been shown for kinases. Posttranslational modifications of tubulins are complex and have been compared to the histone code. Surprisingly, little is known about the roles of these modifications in cancer. The very recent discovery and characterization of the TTLL family is a promising field for new discoveries

We show that human TTLL12 is expressed in the proliferating layer of benign prostate. Expression increases during cancer progression to metastasis. It is highly expressed in many metastatic prostate cancer cell lines. It partially colocalizes with vimentin intermediate filaments and cellular structures containing tubulin, including midbodies, centrosomes, intercellular bridges and the mitotic spindle. Downregulation of TTLL12 affects several posttranslational modifications of tubulin: detyrosination and subsequent deglutamylation and polyglutamylation (latter two not shown). Overexpression alters chromosomal ploidy. These results raise the possibility that TTLL12 could contribute to tumorigenesis through effects on the cytoskeleton, tubulin modification and chromosome number stability. This study contributes a step toward developing more selective agents targeting microtubules, an already successful target for tumor therapy.

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New synthetic flavones of natural origin as antimalarial agents

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Malaria remains the first world endemic parasitic disease endangering 2.5 x 10^9 humans annually. A dramatic recrudescence of the disease is ongoing due to the increasing resistance of the parasite, mainly *Plasmodium falciparum*, to existing drugs. These developments and the difficulty of creating efficient vaccines underline the urgent need for new antimalarial drugs. Natural products have played a key role throughout the history of antimalarial drug discovery. In this context, we tested the antimalarial activity of lanaroflavone, a biflanonoid isolated from the methanol extract of the aerial part of *Campnosperma panamense* (Anacardiaceae), an endemic tree species of Colombia[1,2]. This natural product showed good *in vitro* antimalarial activity but was inactive in an *in vivo* animal model.

In this work, 10 new simplified synthetic analogs of lanaroflavone were tested for the first time against blood stage culture of *P. falciparum* 3D7 (Africa, chloroquine sensitive) and 7G8 (Brazil, chloroquine resistant) strains. We used immunoenzymatic technique based on the *Plasmodium* Lactate Deshydrogenase (pLDH) production to evaluate the inhibition of parasitic growth and to determine the target stage throughout the erythrocytic cycle of *Plasmodium*. Haemotoxicity and cytotoxicity was also evaluated.

Out of the 10 compounds tested, MR27770 and MR27786 exhibited interesting antimalarial activities against the two strains of *P. falciparum* with IC_{50} values below 200nM. We noted no hemolytic effect *in vitro* on red blood cells and no cytotoxicity on cultured hepatic mouse cells Hepa 1-6 and on Normal Human Dermal Fibroblasts (NHDF), with selectivity indexes above 1000.
The promising analogs will be evaluated for *in vivo* antimalarial activity in *P. berghei* infected mice.
This work supports the investigation of natural products for drug discovery.

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Antiproliferative and apoptotic effects of black currant juice (*Ribes nigrum*) on lymphoblastic leukemia cells

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Polyphenols are a group of naturally occurring compounds widely present in fruits and vegetables in human daily diets. These compounds have been reported to show anticancer and anti-mutagenic activities. Black currant juice (*Ribes nigrum*) is a rich source of polyphenols containing about 4.1 g/L. The aim of the present study was to determine whether black currant juice inhibits the proliferation of acute lymphoblastic leukemia cells (Jurkat cells) and, if so, to determine the underlying mechanism. Black currant juice inhibited the proliferation and induced cell cycle arrest in G2/M phase that led to a strong apoptotic effect. Cell cycle arrest and apoptotic effects were accompanied by an upregulation of p73 and caspase-3, and down-regulation of UHRF1. These findings indicate that black currant juice is a strong inducer of apoptosis in Jurkat cells. Mechanistic studies revealed that black currant juice significantly increased the formation of reactive oxygen species (ROS). The formation of ROS was accompanied with a strong upregulation of stress-related kinases (pJNK, p38 MAPK, pERK and pAkt) in a time-dependent manner. Intracellular inhibitors of ROS such as MnTMPyP, N-acetylcysteine and PEG-catalase inhibited the black currant juice-induced formation of ROS and upregulation of stress-related kinases. The role of black currant juice-induced formation of ROS and upregulation of stress-related kinases in apoptosis still remains to be explored. These results indicate that black currant juice is a potent inducer of apoptosis in acute lymphoblastic leukemia cells and, hence, may be of potential in leukemia therapy.

Trefoil Factor 1 (TFF1) molecular function in breast cancer

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Breast cancer is a major public health problem. Two thirds of mammary tumors express estrogen receptors (ERs) and are hormone-dependent. The TFF1 gene (previously named pS2), was isolated in the hormone-dependent breast cancer cell line MCF-7 as a direct transcriptional target of ER. In breast cancer, TFF1 is regulated by estrogen and can be used as an indicator for the response to anti-hormonal therapy and more favorable outcome (1).

The TFF1 gene encodes a secreted protein of 60 amino acids normally expressed and secreted by epithelial mucus cells lining the stomach. This protein acts as a guardian of the integrity of this tissue (2). Currently, it is well established that TFF1 exerts beneficial effects on gastrointestinal tract (3). However its role in breast cancer remained controversial. While most clinical studies conclude that its expression in breast cancer is associated with a favorable outcome (4), other studies suggest that high levels of TFF1 expression may promote cancer cell invasion and may be involved in establishing distant metastasis (5) (6). We recently performed TFF1 gain- or loss-of-function experiments in four human mammary epithelial cell lines and showed that although forced TFF1 expression stimulated the migration and invasion in the four cell lines, TFF1 expression did not modify anchorage-dependent or -independent cell proliferation. By contrast, TFF1 knockdown in MCF7 enhanced soft-agar colony formation. This increased oncogenic potential of MCF7 cells in the absence of TFF1 was confirmed in vivo in nude mice. Altogether, our studies show that TFF1 does not exhibit oncogenic properties, but rather reduces tumor development (7).

To further address the function of TFF1 in mammary carcinomas at a mechanistic level, we are producing TFF1 recombinant protein in an insect cell system. We successfully set up a protocol to purify secreted TFF1 in high amounts. We must establish that this recombinant protein can rescue TFF1 loss of function in mammary epithelial cell lines and use this protein to isolate a specific receptor and to determine in which signaling pathway TFF1 is directly involved.

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PCBIS : an academic drug discovery platform with industrial standards

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High Throughput Screening (HTS) is the technology which best facilitates the search of new molecules with the potential of becoming the drugs of tomorrow. Until recently this expensive technology was only available in pharmaceutical companies.

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Characterization of the first murine model for autosomal recessive centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of congenital disorders characterized by hypotonia and typical skeletal muscle biopsies showing small rounded fibres with centralized nuclei. Three forms have been documented: the X-linked form with mutations in phosphoinositide phosphatase myotubularin (MTM1), an autosomal dominant form with mutations in dynamin 2 (DNM2) and our group has recently identified mutations in amphiphysin 2/BIN1 in patients with autosomal recessive centronuclear myopathy, including two mutations leading to a premature stop codon. These mutations were previously shown to have an impact either on BIN1 tubulation properties or on interaction with protein partners. The aim of our research is to better understand the role of amphyphysin2/BIN1 in healthy muscle and in the pathology of CNM. We are characterising constitutive (CMV) and muscle-specific (HSA) BIN1 knockout (KO) mice lines generated by targeted homologous recombination in ES cells, for exon 11 (a muscle specific exon encoding a phosphoinositide binding motif) and exon 20 (encoding part of the SH3 domain). The first deletion is expected to disrupt the regulation of BIN1 by PIs and thus potentially the link with MTM1. Deletion of exon 20 should potentially interfere with BIN1 protein interactions, including the interaction with its physical binding partner dynamin 2. The deletion of exon 20 deletion may mimic and behave similarly to the BIN1 premature stop codon mutations found in some CNM patients. Although CMVexon11 KO mice seem relatively healthy and live as long as their heterozygous littermates, muscle biopsy analysis has revealed increased centralization of nuclei, which is one of the main features of CNM. We are currently performing behavioural studies and additional immunohistochemical analysis to characterize the CNM features observed further. Considering the second construct, both total (CMV) and muscle-specific (HSA) KO of exon 20 were perinatally lethal. The homozygote mice are not grossly different from other littermates and the cause of death is currently being investigated. Characterization of the first animal model for autosomal recessive CNM will give us a better comprehension of the pathological mechanisms leading to this disease.