10 Cancer Pharmacology

10.001 Paclitaxel reduces tumor growth through reprogramming M2-like tumor-associated macrophages (TAMs) to a pro-inflammatory M1 phenotype via TLR4 activation. Wanderley CW[1], Colón DF[2], Luiz JP[2], Oliveira FF[2], Viacava PR[2], Leite CA[2], Pereira JA[2], Silva CM[1], Silva CR[2], Silva RL[2], Speck-Hernandez CA[2], Mota JM[2], Alves-Filho JC[2], Lima-Júnior RC[1], Cunha TM[2], Cunha FQ[2]
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Introduction: Paclitaxel (PCX) is an antineoplastic agent widely used to treat several solid tumor types. The primary PCX mechanism of action is based on microtubule stabilization inducing cell cycle arrest. Here, we use several tumor models to show that PCX not only induces tumor cell cycle arrest but also promotes antitumor immunity, reprograming M2-like tumor-associated macrophages (TAMs) to antitumor M1-profile via TLR4 activation. **Methods:** A bioinformatical analysis of an online database containing biopsy transcripts from patients with ovarian cancer obtained before and after 3 cycles of PCX therapy (GSE15622) was performed with focus on immunological signatures. Next, bone marrow-derived macrophages from wild-type (WT) or TLR4 knockout mice were polarized to M2 profile with IL-4 (10 ng/mL) and then stimulated with PCX (10, 30, 100 μM) or LPS (100 ng/mL) as control. After 48h, M1 and M2 markers of polarization were measured by cytometry and ELISA. Furthermore, mice WT, TLR4 knockout or with a conditional deletion of TLR4 on macrophages (LysM-Cre$^{+/-}$/TLR4$^{fl/fl}$) were inoculated with murine breast cancer (4T1) or melanoma (B16) cell lines and treated with saline or PCX. Following, the tumors were harvested, and the TAMs isolated by standard percoll (positive selection) for phenotype analysis by qRT-PCR. **Results:** Gene expression dataset analysis indicated an enrichment of genes linked to M1-profile (IFNy stimulated macrophages) in tumor biopsies from PCX treated patients vs. control. Second, in *in vitro*, PCX reprogrammed M2-polarized macrophages to the M1 phenotype in a TLR4-dependent manner, similarly to LPS. Once that M2-cells subsequently stimulated with PCX or LPS displayed reduced M2 markers (CD206 and IGF-1) and increased pro-inflammatory cytokine secretion (TNF, IL-6 and IL-12), an effect that was not reproduced in TLR4-deficient macrophages. Accordingly, in *in vivo* condition, was observed that PCX played an antitumor effect through modulating the TAMs profile. Gene expression analysis of TAMs from tumors of PCX treated mice showed reduced expression of M2 markers (cd206, relma, mmp9 and arg1) and up-regulation of M1 linked genes (Il12, inos and Il6). Additionally, this effect was associated with the tumor growth reduction induced by PCX. To confirm whether the PCX immune mechanism involved TAMs polarization via TLR4, we used mice selectively lacking TLR4 on macrophages by generating conditional LysM-Cre$^{+/-}$/TLR4$^{fl/fl}$ mice. Strikingly, the antitumor effect of PCX was significantly reduced in LysM-Cre$^{+/-}$/TLR4$^{fl/fl}$ mice. **Conclusion:** These findings indicate that PCX skews TAMs towards an M1 immunocompetent profile via TLR4, which might contribute to the antitumor effect of PCX and provide a rationale for new combination regimens comprising PCX and immunotherapies as an anticancer treatment. **Founding:** FAPESP & CNPq, **CEPA:** 56/2016. **License number of ethics committee**: 56/2016 **Financial support**: FAPESP & CNPq

10.002 Fruticuline A has antineoplastic effect by inhibiting NFkB pathway *in vitro* **and** *in vivo*.
Corso CR[1], Stipp MC[1], Silva LM[2], Ramos EAS[3], Klassen G[3], Oliveira CS[4], Stefanello MEA[4], Elferink RO[5], Acco A[1] [1]UFPR – Farmacologia, [2]Univali – Ciências Farmacêuticas, [3]UFPR – Patologia, [4]UFPR – Química, [5]University of Amsterdam –Medical Center
Introduction: Cancer is a major health problem with high mortality rates. Researchers have been studying new agents for the treatment of cancer, and the natural compounds stand out due to their wide biological activity, as the *Salvia lachnostachys* specie. It has been recently demonstrated by our group that the ethanolic extract from leaves of *Salvia* (EES) has antitumor effect *in vivo*. The majority compound in EES is the diterpene Fruticuline A (Fruti). Thus, this study aimed to investigate the antitumor effect and correlated mechanisms of Fruti in cancer cell lineages and in Solid Ehrlich Carcinoma in mice. **Methods:** *In vitro*: MCF-7 and HepG2 cells were incubated with Fruti (10 – 50 µM) and analyzed for proliferation (WST-1 assay, Cyclin D1 levels), oxidative stress (GSH and ROS levels), angiogenesis (Vegf levels), signaling pathways of carcinogenesis (pJUNK, STAT3, pSTAT3 and NFκ1 expression), and cellular death pathways: aptoptosis (Caspase 3/7 activity and Bcl-2 levels) and necroptosis (Ripk1 and TNF-α levels, and WST-1 assay in the presence of Necrostatin-1, inhibitor of Ripk1). *In vivo:* Ehrlich cells were inoculated subcutaneous in the right pelvic member (2^x10^6 cells/mice) of female Swiss mice (CEUA/BIO-UFPR; n. 879). Animals were treated with vehicle (10 mL kg^{-1}, p.o.), Fruti (3 mg kg^{-1}, p.o.) or methotrexate (2.5 mg kg^{-1}, i.p.) for 21 days. Tumor tissue was analyzed for proliferation (*Cyclin D1* levels), apoptosis (*Bcl-2* levels), inflammation (NAG, TNF-α, IL-10, IL-6 and IL-4 levels), angiogenesis (*Vegf* levels), NFκB pathway (*Rela tx2* and *IkBa* levels), and cellular death (tumor histopathology and *Ripk1* levels). **Results and Discussion:** *In vitro:* Fruti (50 µM) reduced MCF-7 and HepG2 cell proliferation, by the reduction on Cyclin D1 levels (76 and 74%, respectively), induced apoptosis in HeG2 cells and reduced Bcl-2 gene expression in MCF-7 cells (66%). In addition, Fruti decreased NFκB1 gene levels in 63% and induced necroptosis by increase in Ripk1 (255%), confirmed by the reversion of the antiproliferative activity in the presence of Necrostatin-1. However, Fruti did not change oxidative stress parameters, Vegf levels and TNF-α. *In vivo:* Fruti prevented in 51% the tumor development (at day 21 of treatment) and reduced *Cyclin D1, Bcl-2* and *Rela tx2* gene levels (48, 54 and 32%, respectively), but not *IkBa*. Furthermore, Fruti induced necrosis in tumor tissue and increased *Vegf*, NAG and TNF-α levels (58, 45 and 79%, respectively) and reduced IL-10 (68%). Nevertheless, *Ripk1* gene was not detected in tumor tissue. **Conclusion:** Collectively, Fruti has antitumor effect through the inhibition of NFκB pathway, reducing Cyclin D1 and Bcl-2 levels. *In vitro* the apoptosis and necroptosis pathways are involved in the cellular death, whereas *in vivo*, due to the long-term treatment and tumor cell plasticity, cells go to necrosis by the increase in tumor inflammation and reduction on angiogenesis. Thus, Fruticuline A has a combination of action mechanisms upon tumor cells and represents a promise molecule for drug development to cancer treatment, mainly for solid mammary and hepatic tumors. Support: CAPES and CNPq (Process 307977/2015-3). **License number of ethics committee**: 879 **Financial support**: CAPES and CNPq (Process 307977/2015-3)

10.003 Pharmacological potential of Etoricoxib in breast cancer. Gonçalves RM, Agnes JP, Delgobo M, Santos VW, Zanotto-Filho A UFSC – Farmacologia

Introduction: Breast cancer is the most frequent cancer in Brazilian women. It is a very heterogeneous disease, which displays a variety of clinically relevant histological and molecular patterns. Recent studies have pointed that a pro-inflammatory microenvironment associated with COX-2 overexpression in mammary tissues acts as an important component of breast cancer oncogenesis and progression. In this study, we tested the effect of the selective COX-2 inhibitor etoricoxib upon proliferation, invasion and angiogenesis in breast cancer models *in vitro* as well as breast cancer oncogenesis and progression *in vivo*. **Methods:** *In vitro:* the effect of etoricoxib on COX-2 activity in MDA-MB231 and MCF-7 cell lines was determined from quantification of PGE2 (prostaglandin E2) by ELISA. Invasion was determined by transwell assay. Angiogenesis was inferred from quantification of vessel-like CD31-positive structures formed by endothelial cells. MTT assays were used to monitor cell viability. *In vivo:* female Swiss mice (30 days-old) (CEUA number: 3722260417) were divided in two different experimental approaches: i) Breast cancer oncogenesis assay: mice (N=7/group) were exposed to 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis and then treated with Etoricoxib (5mg/Kg/day) by gavage for a maximum 120 days or until humane endpoints were achieved. The tumor latency, multiplicity, time-to-progression and survival were evaluated. ii) engraftment assay: mice (N=10/group) were implanted with Ehrlich tumor cells ($3x10^6$ cells/mice) into the mammary gland and, after detection of palpable tumors, treated with Etoricoxib (5mg/Kg/day) by gavage for additional 15 days. Tumor volume was evaluated using a caliper. **Results:** *In vitro:* Etoricoxib inhibited PGE2 production from 1 µM (45%) with maximal inhibition (>90%) from 10 µM in MDA-MB231 cells. In transwell assay, MDA-MB231 cells invasion was 35 % reduced with 10 to 25 µM Etoricoxib in a dose-independent manner. In addition, conditioned medium from etoricoxib-treated MDA-MB231 showed reduced angiogeneic activity compared to conditioned medium from untreated cells. In contrast, breast cancer cells proliferation was not affected by etoricoxib up to 50 µM. *In vivo:* In the DMBA model, etoricoxib did not change the tumor latency. However, once a tumor was detected, the time to progression towards the survival endpoint was longer in etoricoxib-treated mice, indicating a prolonged time to progression. In Ehrlich's tumor bearing mice, etoricoxib treatment reduced tumor growth kinetics. **Conclusion:** This study demonstrates that Etoricoxib is able to inhibit angiogenesis elicited by conditioned medium from breast cancer cells as well as to attenuate cell invasion whereas none effect upon cell proliferation was observed *in vitro*. *In vivo* models showed that etoricoxib delayed tumor growth and disease progression. Future work will address the impact of etoricoxib upon tumor inflammatory microenvironment and angiogenesis in breast cancers *in vivo*. **References:** Bowers LW, deGraffenried LA. Curr Pharmacol Rep; v 1, p. 336-345, 2015; Dubois RN, et al FASEB J; v. 12, p. 1063–1073, 1998. Acknowledgements: Brazilian funding agencies CAPES and CNPq for providing fellowships for RMG, JPA and MD. **License number of ethics committee**: 3722260417

10.004 The role of GPR40/FFAR1 in a mouse model of cancer-induced cachexia. Freitas RDS[1,2], Costa KM[2], Campos MM[2,1,3] [1]PUCRS – Toxicologia e Farmacologia, [2]PUCRS – Medicina e Ciências da Saúde, [3]PUCRS – Odontologia

Introduction: Cancer cachexia (CC) is a multifactorial syndrome leading to muscle atrophy, fat loss and low quality of life (Fearon et al. *Lancet Oncol*, 12: 489, 2011). N-3 fatty acids (FA) supplementation is often used for CC care, but scientific evidence on its use is still lacking (Lavriv et al. *Clin Nutr*, 25: 18. 2018). Docosahexaenoic acid and α-linolenic acid are endogenous agonists of the free fatty acid receptor 1 (GPR40/FFAR1). Our previous data revealed beneficial effects for both FA in a mouse CC model (Freitas et al. Annals of the XXXII FESBE Meeting, 2017). This study evaluated the effects of the selective synthetic GPR40/FFAR1 agonist GW9508, in a mouse model of CC. **Methods:** The local Ethics Committee (PUCRS/CEUA 7164) approved the experimental protocols. Lewis lung carcinoma (LLC) cells were used for CC induction. LLC cells were cultured under standard conditions. On day 0, the cells were resuspended at 5 x 10^6/100 µL of phosphate-buffered saline (PBS), and injected subcutaneously on the right flank of C57/BL6 male mice (20-15g; 8-10 weeks old; n=8/group). Mice were distributed into six groups: tumor-free control + PBS; tumor-free control + GW9508 2 mg/kg; tumor free control + GW9508 8 mg/kg; LLC + PBS; LLC + GW9508 2 mg/kg; LLC + GW9508 8 mg/kg. From day 7 to 21, GW9508 was dosed every other day, subcutaneously. Ambulatory movement, traveled distance, speed, rearing numbers, motor coordination and grip strength were evaluated on day 21. After euthanasia, the weight of muscle and fat tissues, spleen, tumor-free carcass and tumor mass was registered. Blood was collected to analyze hematocrit (Ht) levels. **Results:** LLC-mice had a decrease of ambulatory movement (-22 ± 14 %), rearing numbers (-19 ± 14%), speed (-31.3 ± 8%) and traveled distance (-28 ± 13 %). Both doses of GW9508 (2 and 8 mg/kg) failed to recover the behavioral changes. Motor coordination and grip strength were diminished by CC induction (-8 ± 4%;-16 ± 2%), and either parameters were rescued by GW9508 (8 mg/kg) (+24.4 ± 3 %; +14.4 ± 9 %). LLC-non treated mice showed a small reduction of tibialis anterior (TA) weight (-8 ± 6%;-13.2 ± 7%), and GW9508 (8 mg/kg) partially recovered the TA atrophy (+27 ± 13 %). Additionally, LLC-mice showed a reduced weight of retroperitoneal fat (rWAT), intrascapular fat (isWAT) and epididimal fat (epWAT) (-51.3 ± 7%;-40.7 ± 6%;-39 ±7%, respectively). Administration of GW9508 (8 mg/kg) recovered the fat weight loss toward to control values (+66.4 ± 31%; +19.3 ± 12%; +102.7 ± 26%). Tumor free carcass weight was reduced in LLC-mice (-16 ± 2 %), and GW9508 (8 mg/kg) improved this effect (+8.5 ± 4%). GW9508 treatment (8 mg/kg) also reduced tumor weight (-38 ± 8%).CC induction caused reduced Ht levels (-41.2 ± 5%), associated with splenomegaly (+627 ± 68% of spleen weight). GW9508 (8 mg/kg) partially prevented both CC-related alterations (+17 ± 7 % and -41 ± 8%). GW9508 did not display any effect *per se*. **Conclusion:** GPR40/FFAR1 activation presented beneficial effects in LLC-cachexia mouse model. Further analyses are in progress to investigate more thoroughly the role of this receptor in the CC pathogenesis. **Financial Support:** CAPES, CNPq, PUCRSINFRA #01.11.0014-00. **License number of ethics committee**: 7164

10.005 Neutrophil depletion prevents ifosfamide-induced hemorrhagic cystitis in mice. Silva CM[1], Dornelas-Filho AF[1], Pereira VBM[1], Wong DVT[1], Nobre LMS[1], Melo AT[1], Wanderley CW[1], Alencar NMN[1], Almeida PRC[2], Cunha FQ[3], Lima Júnior RCP[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Patologia e Medicina Legal, [3]FMRP-USP – Farmacologia

Introduction: Ifosfamide is an antineoplastic drug that is commonly used to treat gynecological and breast cancers. Hemorrhagic cystitis is a common side effect associated with ifosfamide injection that affects 6-50% of patients depending on dose intensity. Here, we investigated the role of neutrophils in the inflammatory process associated with ifosfamide-induced hemorrhagic cystitis. **Methods:** Male C57BL/6 mice (n=8/group) received isotype antibody as a control or anti-Ly6G antibody (500 µg/mouse, once daily for 2 days) for neutrophil depletion. Neutrophil (CD11b and Ly6G markers) depletion was confirmed by flow cytometry. Then, the animals were injected with saline (5 ml/Kg, i.p.) or ifosfamide (400 mg/Kg, i.p.) and deprived of access to water. The mice were sacrificed 12 h after ifosfamide injection and the bladders were harvested for evaluation of bladder wet weight, neutrophil infiltration (MPO assay), cytokines levels by ELISA and histopathology. **Results:** Ifosfamide significantly increased bladder wet weight 166% (52 ± 9.5 mg/20g body weight), neutrophil accumulation (1269 ± 288.3 neutrophils/mg of tissue) and IL-6 levels (220 ± 55.3 pg/mg), compared to the saline group (bladder wet weight: 19.6 ± 0.8 mg/20g body weight; neutrophil infiltration: 167 ± 17.1 neutrophils/mg of tissue; IL-6 levels: 0.7 ± 0.4 pg/mg, $P<0.05$). Mice injected with anti-Ly6G antibody showed reduced bladder wet weight (33.5 ± 1.4 mg/20g of body weight), neutrophil infiltration (3688 ± 467.6 neutrophils/mg of tissue) and IL-6 levels (156.4 ± 23.7 pg/mL) in comparison with Ifosfamide-injected group (bladder wet weight: 42.5 ± 1.4 mg/20g of body weight; neutrophil infiltration: 7817 ± 622.6 neutrophils/mg of tissue and IL-6 levels: 468.5 ± 72 pg/mL, respectively). Bladder histopathological injury was also significantly attenuated in neutrophil-depleted mice. Anti-Ly6G alone caused no tissue damage ($P>0.05$ vs. saline group). **Conclusion:** Neutrophils play a critical role in the pathogenesis of hemorrhagic cystitis. **Financial support**: CNPq, CAPES. CEPA: 83/2014 **License number of ethics committee**: 83/2014

10.006 Influence of melatonergic system in human bladder cancer proliferation. Quiles CL, Muxel SM, Cordoba-Moreno MO, Kinker GS, Fernandes PA, Markus RP IB-USP – Fisiologia

Introduction: Melatonin, known as the hormone darkness, produced and released by the pineal gland under the command of the suprachiasmatic nucleus (Reiter, Mol Cell Endocrinol, 79: 1, 1991) is also synthesized by extra-pineal sources (Markus et al., Br J Pharmacol., 2017. doi: 10.1111/bph.14083). Defense cells synthesize melatonin on demand, while cells located in the gastrointestinal tract synthesize in a tonic manner. Recently, it was shown that melatonin synthesized by glioma cells impairs invasion and proliferation and could be a prognostic factor for patient survival (Kinker et al., J Pineal Res, 60: 84, 2016). Indeed, a genic index based on the expression acetylserotonin O-methyltransferase (ASMT) and cytochrome P450 - 1B 1 (CYP1B1), enzymes involved respectively in the synthesis and degradation of melatonin, to genomes deposited in the TCGA was a predictive factor of survival (Kinker, J Pineal Res, 60: 84, 2016). Patients were segregated in low or high survival rate independent of sex, age or histological classification, and the same output was observed when the genic index ASMT/CYP1B1 was applied for patients with bladder carcinoma. The aim of this study was evaluating the expression of the genes and the response to endogenous and exogenous melatonin in two cell lines of urothelial carcinoma cells. **Methods:** Two bladder carcinoma cell lines from the Cell Bank Rio de Janeiro were used: T24 (high-grade), and 5637 (grade II). For both cell lines, gene expression of ASMT and CYP1B1 were evaluated by qPCR using SYBR Green PCR Master Mix (Applied Biosystems, USA). The evaluation of the effects of the melatonergic system in cell proliferation was performed with MTT method as previously described (Kinker, J Pineal Res, 60: 84, 2016), using melatonin (1nM to 1microM) and luzindole (1pM to 100pM), both obtained from Sigma-Aldrich. The results were expressed as the percentage of cell proliferation in relation to the amount of vehicle for each concentration. Data were analyzed by independent t-test and linear regression. **Results:** The ASMT/CYP1B1 index was 25 times lower to T24 than to 5637 cell line, suggesting the first one produces less melatonin than the second one. Surprisingly, blocking melatonin receptors with luzindole, the lower concentration (1 pM) reduced the proliferation in 14% (P=0.04; t=2.29), 10 pM had no effect and 100pM tended to increase T24 proliferation. The statistical significance of the results was evaluated by testing the regression of the dose-response curve (luzindole x proliferation; $F_{1,16}$=8.207; P=0.011). As luzindole has a higher affinity to MT2 receptors (15.5 times), this dual effect is probably dependent on melatonin the expression of melatonin receptors. Melatonin (1nM and 1 microM) did not change the proliferation of T24 cells. Regarding 5637 cell line, neither luzindole nor exogenous melatonin interfered in cell proliferation, strongly suggesting that this cell line does not express melatonin receptors. **Conclusions:** The relevance of the present study is showing that melatonin effect on cancer cell lines is mediated by GPCRs melatonin receptors, in a complex manner. The therapeutic use of melatonin requires more specific analogs, as this indolamine per se can have a dual effect. **Financial support**: FAPESP (grant 2013/13691-1), CLQ PhD fellow FAPESP (2017/10291-3), RPM research fellow CNPq.

10.007 Evaluation of antitumor activity and toxicological profile of compound CPBMF-223, a novel inhibitor of human thymidine phosphorylase. Sperotto NDM[1,2], Roth CD[1], Bergo PHF[1], Freitas TF[1], Campos MM[3,2], Bizarro CV[1], Santos DS[1], Machado P[1], Rodrigues VS[1], Basso LA[1,2]
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Introduction: Human thymidine phosphorylase (HTP) also known as platelet-derived endothelial-cell growth factor is a nucleoside metabolism enzyme involved in pyrimidine salvage pathway. This enzyme and its products trigger angiogenic and anti-apoptotic activities, and increases in their levels are associated with cancer aggressiveness (Elamin, Cancer Microenviron, 9, 33, 2016). The aim of this study was to evaluate cytotoxicity, genotoxicity, and antitumor activity of CPBMF-223, a new inhibitor of HTP. **Methods:** Two cell viability methods, MTT and neutral red uptake assay, were employed after incubations with CPBMF-223 and the different cell lines. For genotoxicity assessment, the comet alkaline assay was performed in HepG2 cells after incubation with CPBMF-223. For the tumor xenograft model, U-87 MG cells (glioblastoma multiform cells, GMB) were implanted into the flank of nude mice. The animals were randomly divided into 4 groups: Control, temozolomide (TMZ), CPBMF-223 and a combination of TMZ plus CPBMF-223. The intraperitoneal treatments were given for 5 days/week for 2 cycles, at the dose of 50 mg/kg/day. Tumors were collected for immunohistochemistry (IHC) and histological analysis. **Results:** Cytotoxicity evaluations showed that incubation of CPBMF-223 (concentrations up to 500 µM) with normal and tumor cells did not significantly affect cell viability by using the two methodologies. Our results showed that CPBMF-223 did not cause DNA-strand breaks even in the higher concentration tested, 1,000 µM. In the *in vivo* model the tumor growth rate was reduced by the three treatment groups when compared to the control. CPBMF-223 and combination groups reduced VEGF (Vascular Endothelial Cell Growth Factor, angiogenesis marker) immunolabeling and numbers of blood vessels; moreover, CPBMF-223 alone also reduce CD31 (vascular endothelium marker) staining when compared to the control. The TMZ group, even having a reduced tumor growth, did not show any difference in the histochemical analysis. The immunostaining for Ki-67 (cell proliferation marker), or capase-3 (apoptosis marker) was not significantly different among the experimental groups. Importantly, tumor-bearing nude mice treated with CPBMF-223 did not show significant body weight alterations. All the experimental procedures were approved by the Local Ethics Committee (8059, PUCRS). **Conclusion:** The results presented herein indicate the favorable safety profile for CPBMF-223 as revealed by the cytotoxic and genotoxic *in vitro* evaluations. The lead compound CPBMF-223 was able to significantly reduce tumor growth when administered to mice. The reduction in tumor volume growth was comparable to that presented by TMZ, a first-line drug for GBM treatment. IHC and histological results supported the capacity of CPBMF-223 to act on tumor angiogenesis. Further studies to confirm the *in vivo* safety of this molecule are underway. **License number of ethics committee**: 8059 **Financial support**: BNDES, CNPq, Capes, Fapergs

10.008 Versican silencing modulates cytokines production and epithelial-mesenchymal transition in trophoblast cells. Tenório LPG, Tanabe ELL, Boteho RM, Silva ALM, Gonçalves CM, Pires KSN, Santos JC, Borbely KSC, Borbely AU UFAL – Ciências Biológicas e da Saúde

Introduction: The placenta is a unique immunological site, responsible for maternal tolerance to the allogeneic fetus and for maternal and fetal defense against possible pathogens and fetal trophoblast cells are critically involved in successful placental development. Likewise, immune cells, trophoblast cells express different receptors from the innate immune response, such as Toll-like receptors (TLR), which are known to bind to danger-associated molecular patterns (DAMP). Amid a plethora of existing DAMPs, versican increased expression has been linked to several inflammatory diseases, as it can link to TLR-2, induce NFkB translocation and increase the production of pro-inflammatory cytokines, as well as increase invasion in cancer cells. Therefore, our goal was to analyze if versican silencing in invasive trophoblast cells can decrease the production of pro-inflammatory cytokines and reduce their epithelial-mesenchymal transition (EMT). **Methods:** First trimester-derived HTR-8/SVneo cells were employed for versican gene silencing and its efficiency was accessed by RT-PCR, immunofluorescence and flow cytometry for versican V0, V1, V2 and V3 isoforms. Supernatants were collected after 24h and ELISA and flow cytometry bead array for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IFN-γ and TNF-α. HTR-8SV/neo cells were also analyzed for falloidin, cytokeratin, and vimentin stainings to evaluate cytoskeleton organization. Invasion through fibronectin-coated Transwell bipartite chambers was analyzed. Also, the expression of epithelial-mesenchymal markers, such as TGF-β and ZEB2 were analyzed by RT-PCR. **Results:** Versican V0 and V1 isoforms were expressed in both cell lines and versican silencing was effective. HTR-8SV/neo cells silenced for versican increased IL-4 and IL-13 secretion, while decreased IL-1β, IL-2, IL-5 and IL-6 secretion. Falloidin staining showed actin depolarization at cell periphery with reduction of vimentin expression. The invasion assay of versican-silenced cells had three times less invaded cells than control. Also, cells expressed less TGF-β and ZEB2. **Conclusion:** Versican silencing in trophoblast cells is able to increase anti-inflammatory cytokines and decrease pro-inflammatory cytokines, which are known to negatively affect trophoblast invasion, which was also diminished with reduction of EMT. Altogether, versican silencing exerts a immunomodulatory role in placenta that reduces trophoblast invasion and switch these cells from a more mesenchymal phenotype to a more epithelial phenotype.

10.009 Telocinobufagin exhibits antitumoral effect on human non-small cell lung carcinoma cells. Godoy TM, Godoy TM, Lopes JB, Castelo Branco MTL, Quintas LEM UFRJ – Ciências Biomédicas

Introduction: Na/K-ATPase (NKA) is a plasma membrane protein vital for cell function. Cardiotonic steroids (CTS) are specific ligands of NKA and are recognized as classical inotropic drugs. In recent years, novel therapeutic perspectives have been proposed for CTS. Particularly, *in vitro* and *in vivo* studies report antiproliferative and antitumor activity of some CTS. Nonetheless, no studies have been performed with telocinobufagin (TCB), a bufadienolide present in the parotoid secretion of Rhinella toads and one component of Chan'Su, a traditional Chinese medicine, and also a CTS found endogenously in humans. We have previously shown that TCB was cytotoxic against human colorectal adenocarcinoma HCT8 cell line. Here, we investigated its effect on human lung cancer H460 and on normal human fibroblast HFF1 cell lines. **Methods:** H460 were cultured in RPMI + 10% FBS and HFF1 in DMEM + 15% FBS + L-glu and antibiotics, and cells were serum-starved for 24 h before treatment. Depending on the method, cells were treated with 10, 30, 100, 300 or 1000 nM TCB for 24, 48 or 72 h. In 24-well plates, Trypan blue viable cells were counted in Neubauer chamber and in 96-well plates cell viability was assessed by MTT assay and cell growth was assessed by [3H]thymidine incorporation assay. **Results:** TCB significantly decreased cell number after 24 and 48 h for 100 and 300 nM (29 and 54%, and 26 and 62%, respectively; $p<0.05$, $n=5$) and with 30, 100 and 300 nM TCB after 72 h (43, 51 and 90%, respectively; $p<0.05$, $n=4$). MTT assay showed a significant reduction in viability after 48 h at 100, 300 and 1000 nM (44, 75 and 88%, respectively; $p<0.05$, $n=4$) and decline of [3H]thymidine incorporation was observed after 24 h at 100, 300 and 1000 nM (45, 65 and 88%, respectively; $p<0.05$, $n=3$). Interestingly, TCB treatment at the same time and concentrations did not affect HFF1 in any experiment performed. **Conclusion:** As in HCT8 cells, TCB has an antiproliferative effect in a concentration and time-dependent manner in human lung cancer cells but not in normal human fibroblasts. Ongoing studies aim to characterize whether TCB is cytostatic or cytotoxic to cancer cells and its mechanism of action. **Financial support**: CNPq, FAPERJ, CAPES.

10.010 Cell Surface protein disulfide isomerase inhibition sensitizes melanoma cells resistant to the BRAF inhibitor Vemurafenib. Beyerstedt S, Lopes LR, Machado-Neto JA ICB-USP – Farmacologia

Introduction: Melanoma is the most dangerous form of skin cancer and has the worst prognosis due to its great metastatic and invasive potential and resistance to the current chemotherapy. Most of the patients present the BRAF V600E mutation and acquire resistance to the BRAF inhibitor vemurafenib during the treatment. Cell surface Protein disulfide isomerase (csPDI) is a redox chaperone highly expressed in the surface of melanoma cells. Our group has demonstrated csPDI regulates NADPH oxidase expression and activity in different cell types. Nox4 is also highly expressed in melanoma and responsible for constitutive reactive oxygen species (ROS) production, which is important for tumor cell survival. Vemurafenib causes oxidative stress and this has been associated to resistance development. Thus, targeting redox regulated signaling pathways overactivated in melanoma may represent a new approach in the treatment of this disease. **Goal:** Investigate the effect of csPDI and Nox in the mechanism that enables resistance of melanoma cells to BRAF inhibitors. **Methods:** Human primary melanoma cell lineage SKMEL-28 and its vemurafenib resistant lineage SKMEL-28R were treated for 24 and 48 hours with the antibiotic Bacitracin, the B-RAF inhibitor, Vemurafenib, the flavonoid quercetin-3-rutinoside (Rutin) and diphenyleneiodonium (DPI). Cell viability was analyzed using MTT assay, and survival to rutin treatment was estimated by Trypan blue assay, to ensure that the rutin antioxidant effect was not interfering with the reduction of the tetrazolium salt to formazan. **Results:** The association of a PDI broad inhibitor, bacitracin with vemurafenib decreased cell viability in both cell lineages SKMEL-28 and SKMEL-28R when compared to bacitracin or vemurafenib only. The association of a cell surface PDI inhibitor, Rutin and vemurafenib reduced cell viability in SKMEL-28R when compared to treatment with rutin or vemurafenib only. In order to investigate the role of NADPH oxidases in the resistance to BRAF inhibitors, we treated melanoma cells with DPI. The combination of DPI and vemurafenib decreased cell viability in both lineages when compared to DPI or vemurafenib only. **Conclusion:** The inhibition of cell surface PDI and NADPH oxidase sensitized resistant melanoma cells to treatment with the BRAF inhibitor vemurafenib. Therefore, we propose that cell surface Protein Disulfide Isomerase could represent a novel therapeutic target to treat resistance to BRAF inhibitors in melanoma. **References:** Ribeiro-Pereira C. PLoS ONE; 9. 2014. Lovat PE. Cancer Research; 68; 5363-9. 2008. Janiszewski M. J Biol Chem; 280; 40813-9. 2005. Chapman PB. N Engl J Med; 364; 2507-16. 2011. **Funding acknowledgements:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) **Ethics approval:** This is study was approved by the Ethics Committee on the use of animals (CEUA ICB/USP) and the Ethics Committee on Human Research (CEPSH ICB/USP) **License number of ethics committee**: 917/2017 **Financial support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

10.011 Molecular and cellular mechanisms of pradimicin-IRD in colorectal cancer cells. Almeida LC[1], Bauermeister A[2], Santos EA[3], Moraes LAB[2], Machado-Neto JA[1], Costa-Lotufo LV[1] [1]ICB-USP – Farmacologia, [2]FCFRP-USP – Física e Química, [3]ICB-USP – Biologia Celular e Molecular

Introduction: Most of antineoplastic drugs used in clinical practice are derived from natural products. Great efforts have been applied to identify new compounds to generate novel therapeutic opportunities for the treatment of cancer. In a previous study of our research group, Pradimicin-IRD, a new polycyclic antibiotic derivative from actinobacteria *Amycolatopsis sp*., presented antimicrobial and potential anticancer activity. Our previous results present dose- and time-dependent cytotoxicity activity in HCT-116 cells, with reduction of cell density and viable cells, and IC_{50} in micromolar order. Additionally, it was presented that from 6 hours of treatment, Pradimicin-IRD is capable of induce potential cytotoxic activity; also morphological assay indicates apoptosis. The apoptotic effect of Pradimicin-IRD was confirmed by the induction of apoptotic proteins, such as cleavage caspase-4 and cleavage PARP-1, which can be related to the DNA damage – observed by the induction of yH2AX. The Aims of the present study was to understand the mechanism of action of Pradimicin-IRD in the colorectal cancer cell line HCT-116. **Methodology:** The anticancer studies of Pradimicin-IRD were based on flow cytometry and qPCR with time of treatment of 48 hours and concentrations of treatment of 1.25μM, 2.5μM and 5μM - determined previously. Clonogenic assay was performed with the same concentrations, however with time drug exposure time of 6 and 24 hours (with normalizations of cell number – 2000 cells/well) - also determined previously. Doxorubicin was used as positive control. The results were analyzed with the GraphPad Prism 5, FlowJo and ImageJ softwares. **Results:** Phenotypic characterization of Pradimicin-IRD cytotoxicity shows reduced cloning capacity in 6 hours of treatment without cell normalization [(colony counting: control - 1; 1.25μM – 0.37; 2.5μM – 0.24; 5μM -0.16) (colony area: control – 1; 1.25μM – 0.08; 2.5μM – 0.074; 5μM -0.03)] and 24 hours of treatment with cell normalization [(colony counting: control - 1; 1.25μM – 0.61; 2.5μM – 0.58; 5μM -0.64) (colony area: control - 1; 1.25μM-0.65μM; 2.5μM - 0.6; 5μM-0.53)]. Additionally, apoptosis was observed in the increase of the SubG1 population (control - 9.9%; 1.25μM - 44.3%; 2.5μM - 46.2%; 5μM-58.2%) and G1 phase arrest (control – 46,8%; 1.25μM – 58,2%; 2.5μM – 57,4%; 5μM -60,7%) of the cell cycle. Molecular analysis presents the increase of the RNA marker of DNA damage (p21 – 22-fold) and the reduction of the RNA of cyclins that promote the progression of the cell cycle (cyclin A – 0.4-fold; and cyclin B – 0.3-fold) by Pradimicin-IRD treatment. **Conclusion:** Pradimicin-IRD presents anticancer activity. Cellular and molecular analysis indicates that Pradimicin-IRD potentially induces DNA damage, cell cycle arrest and apoptosis in HCT-116 colorectal carcinoma cell line. New studies focused on the identification of the Pradimicin-IRD DNA damage mechanisms are being conducted by our research group, aiming at a better understanding of the compound. **Financial support**: FAPESP 2015/17177-6

10.012 Industrial residue of *Cyperes articulatus* **L**. (**Cyperaceae**) **rhizomes as a source of antiproliferative compounds**. Silva EBS[1], Barata ES[2], Vieira LQ[3], Castro KCF[2], Almeida JS[4], Sartoratto A[5], Barato LC[6], Moraes WP[4], de Santana MB[4], Pires Moraes TM[1] [1]UFOPA - Saúde Coletiva, [2]UFOPA – Produtos Naturais Bioativos, [3]UFMG – Gnotobiologia e Imunologia, [4]UFOPA – Farmacologia, [5]CPQBA-Unicamp, [6]UFRJ –Pharmacognosy

Introduction: *Cyperus articulatus* L. (Priprioca) is a medicinal plant. Family Cyperaceae traditionally used in traditional medicine. Studies of the essential oil of this species have identified many terpene compounds. However, little is known about the possible uses of solid waste generated by the extraction of essential oil. **Methods:** This study aimed to investigate the chemical composition and to evaluate the antiproliferative activity of the ethanolic extract of solid residues generated by the extraction of essential oil of *Cyperus articulatus* L. in experimental models *in vitro* using peritoneal macrophages (CEUA/UFOPA 07004/2013) of mice and human tumor cell lines. **Results:** The analysis of the chemical composition indicated the presence of monoterpenes, sesquiterpenes and particularly sequiterpenic ketones as main constituents. The results showed that the treatment with ethanolic extract of *Cyperus articulatus* L. reduces the activity of the enzyme arginase and proliferation of cancer cells ($F (5, 11) = 137,6$ $P < 0,0001$, one-way ANOVA with Tukey's multiple comparisons test). The extract also showed no cytotoxicity in macrophages in concentrations between 12.5; 25 and 50 mg/ml ($F (5, 12) = 60,93$, $P < 0,0001$). The results indicated that the extract of *Cyperus articulatus* L. exerts antiproliferative activity ($F (4, 80) = 25995$, $P < 0,0001$). with low toxicity on healthy cells in experimental models *in vitro*. **Conclusion:** The results demonstrate that ethanolic extract of *Cyperus articulatus* L. exhibits antiproliferative activity on tumor cell lines without cytotoxicity on normal cells. **Keywords:** Extract, medicinal plant, cytotoxicity, arginase, antiproliferative, anticancer. **License number of ethics committee**: CEUA/UFOPA 07004/2013 **Financial support**: Universidade Federal do Oeste do Pará - UFOPA; Fundação Amazônia Paraense de Amparo à Pesquisa - FAPESPA.

10.013 Characterization of cytotoxic activity of seriniquinone and its synthetic derivatives. Hirata AS[1], Rezende-Teixeira P[1], Hammons JC[2], Trzoss L[2], Jimenez PC[3], La Clair JJ[4], Fenical W[2], Costa-Lotufo LV[1] [1]ICB-USP – Farmacologia, [2]University of California - Marine Biotechnology and Biomedicine, [3]Unifesp – Ciências do Mar, [4]University of California - Chemistry and Biochemistry

Introduction: Seriniquinone (SQ) is a secondary metabolite isolated from a marine bacterium of the genus *Serinicoccus*. This compound has been shown to regulate the expression of the DCD (dermcidin) gene and to induce cell death by autophagy more potently in melanoma cell lines (TRZOSS et al., 2014). Melanoma is a threatening skin tumor due of its aggressiveness and high resistance to chemotherapy, therefore several therapeutic targets have been explored from the molecular basis of tumorigenesis. The DCD gene, which encodes the dermcidin protein, is a target that has been recently gaining much attention in the perspective of cancer diagnosis and treatment. It is known that this protein has an important role in skin defense. On the other hand, its role in cancer remains poorly understood, with punctual observations related to proliferation, migration, invasion, survival and resistance. Considering that, eleven structural analogs of SQ were synthesized intended at increasing cytotoxicity and selectivity for melanoma cells and, moreover, to improve SQ pharmacokinetic properties. The present work aimed to evaluate the cytotoxicity of these molecules in tumor cell lines. **Methods:** MTT assay (MOSMMAN, 1983) was used to indirectly measure the cytotoxicity of the compounds on three tumor cell lines (HCT-116, SK-Mel-28 and MALME-3M) and to determine their IC_{50}. Resorting to qRT-PCR, the effects of some compounds on dermicidin expression were explored. DNA binding and oxidative stress assays were performed to evaluate quinone-like characteristics of the synthetic SQ analogs. **Results:** The majority derivatives presented a lower IC_{50} towards melanoma cell line MALME-3M, ranging from 0.001nM for 12a/12b and 14a/14b analogs, while for SQ was 0.05nM. Only compound 23 was not active at the tested concentrations (IC_{50} > 15nM). Compound 24, which also displayed good results on solubility and cytotoxicity, increased the dermicidin RNA expression in all cell lines, such as observed for SQ. Compounds SQ, 9a/9b and 24 did show DNA binding neither promoted cellular oxidative stress. **Conclusion:** The SQ synthetic derivatives showed, in general, higher or equivalent cytotoxicity compared to the natural compound in the tested cell lines. Moreover, modulation of dermicidin RNA expression, as shown by SQ, was conserved, indicating a possible interaction of 24 and dermicidin. All the tested substances did not show DNA binding nor oxidative stress effects, which are classic features described for quinones, which excludes such unspecific processes as the mechanism of action of SQ and derivatives. **References:** TRZOSS, et al. *Proc Natl Acad Sci USA*. v. 111. p. 14687. 2014. MOSMMAN, T. J. *Immunol Methods*. v. 65. p. 55.1983. **Acknowledgments:** FAPESP (Process numbers: 2015/17177-6, 2014/50926-0, 2016/10854-5 e 2018/07661-6) and CNPq (INCTBioNat). **Financial support**: FAPESP (Process numbers: 2015/17177-6, 2014/50926-0, 2016/10854-5 e 2018/07661-6) and CNPq (INCTBioNat)

10.014 Chemopreventive effect of *Chuquiraga spinosa* **Less** (**Huamanpinta**) **associated with simvastatin on prostate cancer induced in rats**. Acevedo JA[1], Armas JR[1], Carranza CA[2], Calderon OH[3], Asmat RC[4], Guerrero HJ[1], Torres JC[5] [1]Universidad Nacional Mayor de San Marcos – Ciencias Dinámicas, [2]Instituto Nacional Cardiovascular - Patología, [3]Universidad Nacional Mayor de San Marcos – Farmacognosia y Medicina Tradicional, [4]Universidad Nacional Mayor de San Marcos - Ciencias de la Salud, [5]Universidad Técnica Particular de Loja Ecuador - Ciencias Exactas

Introduction: Prostate cancer is the most frequently diagnosed malignancy and is the fifth leading cause of death in men worldwide. *Chuquiraga spinosa* Less (Huamanpinta) showed cytotoxic effect on tumor cell lines of prostate carcinoma (DU-145). Otherwise, statins are being studied based on proapoptotic and antimetastatic effects. **Objectives:** To determine the supraditive synergism in prevention and management of prostate cancer association between simvastatin and the extract of the aerial parts of *Chuquiragaspinosa* Less. **Biological material and intervention:** During 20 weeks, 56 animals got treatment and they were randomized into groups: 1) Negative control: SSF 2 mL / kg; 2) TCN: Testosterone 100 mg / kg + cyproterone 50 mg / kg + NMU 50 mg / kg; 3) TNC + H250 (Huamanpinta 250 mg / kg); 4) TNC + S40 (Simvastatin 40 mg / kg); 5) + H50 TNC (Huamanpinta 50 mg / kg) + S40; 6) TNC + H250 (Huamanpinta 250 mg / kg) + S40; 7) TNC + H500 (Huamanpinta 500 mg / kg) + S40. **Main outcome measures:** The chemical components of the extract were analyzed on a gas chromatograph and its antioxidant activity with DPPH test (1,1-diphenyl-2-picryl-hidrazil). Hematologic parameters, liver, lipid, PSA, prostate size by ultrasound and pathologic findings were evaluated as indicators. Data were expressed as mean and standard deviation compared by ANOVA and Tukey test with a confidence interval of 95% ($p < 0.05$). Care of animals were taken in according to stipulated ethical standards for experimental animals. **Results:** The ethanolic extract of *Ch. spinosa* has as secondary metabolites; alkaloids, steroids, flavonoids and phenolic compounds; and as volatile metabolites to palmitic acid, galaxolide, gibberellic acid, coumarin. The percentage of antioxidant activity on DPPH was concentration-dependent with 1.0 µg/mL (40.5%), 10.0 µg/mL (70.5%) and 50.0 µg / mL (90.3%). A reduction in the levels of triglycerides was determined in the groups receiving *Ch. spinosa*, being significant ($p = 0.02$) in TCN + H250 group with 87.0 ± 6.5 mg / dL and TNC + H500 + S40 group with 81.6 ± 22.4 mg / dL in regard to the cancer-inducing group (TCN) with 177.3 ± 57.5 mg / dl. No difference was found between PSA levels ($p = 0.71$); in ultrasound the TCN + H500 + S40 group had a prostatic volume of 0.6 ± 0.1 cm^3, which was the lowest of all, being significant compared to TCN + H50 + S40 which was 1.4 ± 0.3 cm^3 ($p < 0.05$). The TCN group showed multiple foci of high-grade prostatic intraepithelial neoplasia (HG-PIN) with the presence of cells in mitosis, unlike the groups TNC + H50 + S40 and TNC + H250 + S40 with few areas of HG-PIN. **Conclusion:** Under experimental conditions, the ethanolic extract of *Chuquiraga spinosa* Less in association with sinvastatin has a chemopreventive effect in prostate cancer through hypolipidemic and antioxidant activity. **Keywords**: Prostate cancer, anti-tumor, *Chuquiraga spinosa*, simvastatin. **License number of ethics committee**: Authorization of the Ethics Committee Faculty of Medicine of San Marcos National Major University: 0260 of 04-04-2016 **Financial support**: Vicerrectorado de investigación de la Universidad Nacional Mayor de San Marcos

10.016 Autophagy inhibition potentiates ruxolitinib-induced apoptosis in JAK2^{V617F} cells. Machado-Neto JA[1], Coelho-Silva JL[2], Scheucher PS[2], Rego EM[2], Traina F[2] [1]ICB-USP – Pharmacology, [2]FMRP-USP – Internal Medicine

Introduction*:* Autophagy is a paradoxical cellular process that works as a double-edged sword in cancer by acting in both tumor cell survival and death. Constitutive activation of tyrosine kinase signaling mimics nutrients and growth factors signal and suppress autophagy. Activation of JAK2/STAT signaling pathway is a hallmark and participates of the pathogenesis of myeloproliferative neoplasms (MPN). Ruxolitinib, a selective JAK1/2 inhibitor approved by the FDA, provides some clinical benefits, but does not lead to complete remission of MPN and fail to eliminate the malignant clone. Considering that autophagy has been described to be involved in cell survival and drug resistance in hematological malignant cells, and that JAK2^{V617F} mutant protein may mimics constitutive growth factors signal, studies to assess the effect of ruxolitinib on autophagy and the effects of autophagy inhibitors may be interesting in MPN. The aim of the present study was to investigate the effects of ruxotilinib treatment alone and in combination with pharmacological autophagy inhibitors in a JAK2^{V617F} cell line. **Methods:** SET2 cell line (harboring JAK2 ^{V617F} mutation) was treated with graded doses of ruxolitinib (100, 300 or 1000 nM). Ruxotinib (300 nM) and autophagy inhibitors (3-methyladenine [1 mM], bafilomycin A1 [10 nM] and chloroquine [20 µM]) were also used in monotherapy or in combination for functional assays. Protein expression and activation was evaluated by Western blot, gene expression by quantitative PCR, autophagy by acridine orange staining (flow cytometry) and LC3BI/II expression, apoptosis by annexin V/PI staining (flow cytometry) and caspase 3 cleavage. **Results:** Ruxolitinib treatment promoted an accumulation of acidic vesicular organelles, which was followed by consumption of LC3BI/II protein, characterizing autophagy induction. The rates of acidic vesicular organelles positive cells were 18%, 38%, 62% and 63% for control cells, ruxolitinib treatment at 100 nM, 300 nM and 1000 nM, respectively. At transcriptional levels, *BCL2* mRNA expression were reduced, while *BECN1* (*beclin 1*) mRNA expression remains stable upon ruxolitinib treatment. Consequently, BECN1/BCL2 ratio was increased by ruxolitinib at 300 and 1000 nM (p<0.05), corroborating induction of autophagy. In SET2 cells, ruxolitinb treatment reduced important autophagy regulators, in a dose-dependent manner, including STAT3^{Y705}, STAT5^{Y694}, mTOR^{S2448}, p70S6K$^{T421/S424}$ and 4EBP1^{T70} phosphorytation and BCL2 expression. All pharmacological inhibitors of autophagy tested were able to significantly suppress the ruxolitinib-induced autophagy, being bafilomycin A1 (reduction of 89%) and chroloquine (reduction of 70%) more efficient than 3-methyladenine (reduction of 10%) (all p<0.05). Importantly, autophagy inhibition significantly increased ruxolitinib-induced apoptosis in SET2 cells (all p<0.05). **Conclusion:** Ruxolitinib induces autophagy in SET2 cells, which potentially represent a mechanism of resistance to apoptosis. The mTOR/p70S6K/4EBP1 and STAT/BCL2 axis were identified to be involved in ruxolitinib-induced autophagy. Our results indicate that combination of ruxolitinib with pharmacological inhibitors of autophagy, especially chloroquine, may be a promissory strategy to improving apoptosis induction in JAK2^{V617F} cells. Our findings add new insights for molecular mechanism involved in ruxolitinib resistance in MPN. **Financial support**: Supported by CNPq and FAPESP.