10. Cancer Pharmacology

10.001 Evaluation of Eugenol anticancer activity by regulation of the oncogenic transcription factor Forkhead Box M1. Wiirzler LAW[1], Aguiar RP[1], Silva-Filho SE[1], Rodrigues PJ[1], Cardia GFE[1], Uchida NS[1], Velázquez-Martínez CA[2], Bersani-Amado CA[1], Cuman RKN[1] – [1]UEM, [2]University of Alberta

Introduction: Genome-wide gene expression profiling of cancers has consistently identified the Forkhead Box M1 (FOXM1) as one of the most commonly upregulated genes in the early stages of carcinogenesis. The FOXM1 plays an essential role in the regulation of a wide spectrum of biological processes, including cell proliferation, cell cycle progression, cell differentiation, angiogenesis, and apoptosis. This transcription factor has been regarded as the “Achilles heel of cancer”, and it represents one of the most promising therapeutic targets for the development of novel anticancer agents. Consequently, several research groups have suggested the need to establish comprehensive and multidisciplinary programs dedicated to study the role and regulation of FOXM1 by chemical compounds. Since the overexpression of FOXM1, and its subsequent elevated transcriptional activity, has been correlated with the direct upregulation of a wide variety of proteins associated to inhibition of apoptosis, such as surviving and Bcl-2, it might indicates correlation between some natural compounds-induced apoptosis effect and downregulation of FOXM1. **Aims**: We conducted some *in vitro* experiments aimed to evaluate the potential cytotoxic activity exerted by eugenol (EUG), a biologically active phenolic component of *Syzigium aromaticum* (the clove) that induces apoptosis in cancer cells by modulating the Bcl-2 family proteins, on three different human cancer cell lines and its potential regulatory effect on FOXM1 protein levels. **Methods**: A series of experiments were conducted to evaluate the effect of EUG on cellular viability on cancer cells by MTT and its potential regulatory effect on FOXM1 protein levels by western blots. **Results and Conclusions**: Our results corroborate with the anticancer effect of EUG on different human cancer cell lines as previously reported in the literature (SKBR3 LD50: 318.6 µM; HT29 LD50: 525.5 µM; and HepG2 LD50: 2090.0 µM). However the EUG did not regulate the FOXM1 protein levels. Then, in this study we demonstrated the anticancer effect of EUG in three cancer cell lines and evidenced that the EUG-induced apoptosis effect is not related to regulation of FOXM1 at protein levels. Further studies must be done to generate information about the mechanism of action of this agent. **Financial Support**: The study was supported by the grant BEX 11387/13-0, CAPES Foundation, Ministry of Education of Brazil, Brasília – DF 70.040-020, Brazil.

10.002 *In vivo* **anti-tumoral effects of simvastatin and pravastatin in a cancer stem cell-rich model of breast carcinoma.** Rennó AL, Alves-Junior M, Souza PC, Souza VB, Latuf-Filho P, Cardelli NJA, Schenka NGM, Schenka AA FCM-Unicamp – Farmacologia

Introduction: Breast cancer is the most frequent malignant neoplasm and the leading cause of death from malignancy among women in Brazil and worldwide. Dimetil-benz(a)anthracene (DMBA) is of the most frequently used carcinogenic substances in the literature, being highly efficient and specific for breast carcinoma induction. Preliminary data from our group indicate that DMBA induced neoplasia is highly enriched in cancer stem cells (CSC), one of the major targets of current drug development research. Simvastatin and pravastatin is a competitive inhibitor of 3-hydroxy-3-methylgluraryl coenzyme A redutase (HMG-coA), widely used in primary and secondary prevention of hyperlipidemic-related cardiovascular diseases. Statins has recently been associated to *in vitro* anti-proliferation actions across a wide variety of malignant cell lines. **Aim**: This study report the antineoplastic effects of simvastatin and pravastatin on a DMBA-induced cancer stem cell-rich model of breast carcinoma. **Methods**: mammary tumor induction was performed by single gavage administration of DMBA (100mg/kg) in virgin female Sprague-Dawely. When the tumors reached approximately 1cm^3, the animals were treated for 14 days with 1mL of soy (DMBA Group, n=10) or simvastatin (50mg/kg/day by gavage, n=6) or with pravastatin (50mg/kg/day in by gavage, n=6). After this 14-day protocol, the animals were euthanized and the tumors removed for macroscopic examination, volume measurement and immunostaining of progenitor and cancer stem cells (CD133, CD24 and CD44). **Results**: the average tumor size of simvastatin treated animals (0,73±0,87cm^3) was significantly ($p<0.01$) smaller than that of control rats (6,52±5,08cm^3) and pravastatin treated animals (4,29±2,12 cm^3). Simvastatin decreased the number of tumors developed (1,4±0,54 tumors/animal $p<0,05$) than control (3,09±1,57 tumors/animal) and pravastatin (2,75±2,06 tumors/animal) groups. Simvastatin was also able to reduce the number ($p<0,05$) of CD133 (70,29±10,04), CD24 (19,93±13,39) and CD44 (8,78±5,5) positive cells than control group (CD133 131±26,22, CD24 51,56±11,31, CD44 19,68±4,28 positive cells) and pravastatin group (CD133 119,79±35,97, CD24 41,56±13,30, CD44 17,68±9,45 positive cells). **Conclusion**: these results indicate that simvastatin is effective as an antineoplastic agent and decrease the CSCs and progenitor cells in the *in vivo* model of mammary carcinoma. **Support**: **CAPES, FAEPEX-UNICAMP, FAPESP. Keywords**: cancer stem cell, breast carcinoma, carcinogenesis model, Sprague-Dawley rat, anti-neoplastic drugs. The experimental protocols were approved by an instructional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP, protocol n. 2335-1)

10.003 Cytotoxic effect of Telocinobufagin on H460 lung cancer cells. Rendeiro MM[1], Azevedo SV[2], Fernandes J[2], Cunha-Filho GSA[1], Noël F[1], Quintas LEM[1] – [1]UFRJ – Farmacologia, [2]UFRJ – Ciências Morfológicas e Fisiológicas

Introduction: Telocinobufagin (TCB) is a cardiotonic steroid (CTS) isolated from *Rhinella scnheideri*'s parotoid gland**.** CTSs are a class of compounds that has Na/K-ATPase (NKA) as their main molecular target in cells. NKA is a plasma membrane protein well-known as an energy-transducing ion pump. Recently it was shown that interaction between CTS and NKA are able to trigger signal transduction pathways that underlie cellular effects like proliferation, differentiation and apoptosis. Many studies also report antitumoral activity of CTSs, although there are very few works about antiproliferative/antitumoral effects of TCB. Our goal was to investigate the effect of TCB on human lung cancer large cell line H460. **Methods**: H460 cells were seeded in 24-well plates and exposed to TCB treatment for 72 h at concentrations of 10, 25, 50, 100, 150 and 200 nM. Phase-contrast light microscopy micrographs were taken before staining with propidium iodide and flow cytometry analysis. Sub-G1 cells were quantified in Cell Quest Analyses Software. Statistical analysis was performed using ANOVA with Newman-Keuls post-test and statistically significant differences were accounted as $p<0.05$ (n=5). **Results**: At all concentrations used micrographs revealed a clear decline in the number of cells attached to the culture plate, but only above 50 nM TCB there was a significant, concentration-dependent, decrease of cell viability measured by flow cytometry (from 18.2 ± 2.1% DNA fragmentation at 50 nM to 84.0 ± 0.7% DNA fragmentation at 200 nM; control: 3.4 ± 0.3%). **Conclusion**: TCB exhibits antitumoral effect in a concentration-dependent fashion, and lack of cell adhesion seems to be one of the first steps. Further studies are in course in order to characterize the type of cell death and signaling pathways involved in this cellular response. **Financial support**: FAPERJ, CNPq, CAPES.

10.004 Effect of simvastatin on MUC1 expression in breast cancer xenografts. Cardelli NJA[1], Souza VB[1], Souza CP[1], Rennó AL[1], Mendonça GRA[1], Anjos D[1], Franchi Jr GC[2], Latuf-Filho P[2], Nascimento FC[2], Resende M[2], Rocha MR[2], Soares F[2], Vassalo J[3], Schenka AA[1] – [1]FCM-Unicamp – Farmacologia, [2]FCM-Unicamp, [3]FCM-Unicamp – Patologia

Background: simvastatin, an HMG-CoA reductase inhibitor, is commonly used in the prevention and treatment of dyslipidemias. Recently, it has been associated to a plethora of antineoplastic effects, the most recent being an inhibitory effect on the expression of cancer stem cells (CSC) markers, in both *in vitro* and *in vivo* contexts. However, this anti-CSC effect has not been properly validated for all CSC markers. **Objective**: Herein, we investigated if MUC1, a well-known CSC antigen and potential therapeutic target, is expressed in MCF7 breast cancer xenografts and if its expression can be affected by an acute treatment with simvastatin. **Methods**: MCF7 cells were grafted in twenty NOD/SCID female mice ($3x10^6$ cells/animal). When the tumors reached $0,5cm^3$, the animals were treated with 1mL of soybean and euthanized 24h later (control group, n=6) or with 40mg/kg of simvastatin (diluted in 1mL of soybean oil) and euthanized 24 or 48h later (simvastatin-24h and -48h groups, respectively, n=6/each). All treatments were given at a single dose, by intragastric gavage. All xenografts were removed for pathologic examination and immunodetection of MUC1. All procedures were approved by the local ethics committee (CEUA-Unicamp; protocol number: 2414-1). **Results**: all MCF7 xenotransplant procedures were successful and resulted in poorly differentiated tumors, with high grade histology, and diffuse infiltration of soft and bone tissue, but no vascular invasion or metastatic spread at the moment of euthanasia. Mean frequency of MUC1+ cells were 89%, 80% and 81% for control, simvastatin-24h and simvastatin-48h groups, respectively. However, no statistically significant differences were found between groups (p=0.4543, ANOVA). **Final Considerations**: since MUC1 levels were not significantly affected by this protocol, we suggest that MUC1 CSCs may be resistant to an acute treatment with simvastin, in contrast to the behavior of CD133+ and CD24-/CD44+ CSCs when submitted to the same challenge, as reported in the literature. This finding may limit the potential of simvastin as an anti-CSC agent, once MUC1+ cells may emerge as a resistant clone during and after treatment with this drug, further indicating the need to validate the effect of simvastatin on other markers, currently considered as typical of the CSC phenotype.

10.005 *In vitro* **evaluation of quinoxaline-derived chalcones associated with standard chemotherapies in oral squamous cell carcinoma.** Mielcke TR[1], Erig TC[2], Chiela EC[3], Kist LW[4], Mascarello A[5], Chiaradia LD[5], Bogo MR[6], Nunes RJ[5], Campos MM[1] – [1]PUCRS – Medicine and Health Sciences, [2]PUCRS – Pharmacy, [3]UFRGS – Hepatology and Gastroenterology, [4]PUCRS – Genomics and Molecular Biology, [5]UFSC – Chemistry, [6]PUCRS – Cell and Molecular Biology

Introduction: Oral squamous cell carcinoma (OSCC) represent a health problem, as indicated by its high incidence worldwide (Lubek et al., J Oral Maxillofac Surg. 71: 1126, 2013). The treatment rely on surgical resection, radiotherapy and chemotherapy. The chemotherapy options are very limited, with serious side effects. Extensive research identified numerous natural compounds with anti-cancer effects, which might present beneficial effects when used in combination with classical chemotherapeutic drugs (Newman et al., J Nat Prod. 75: 311, 2012). We demonstrated before that quinoxaline-derived chalcones, based on the selective PI3Kγ inhibitor AS605240, showed marked antiproliferative effects in glioma cell lines (Mielcke et al., Eur J Med Chem. 48: 255, 2012). **Aims**: This study examined the *in vitro* effects of two different quinoxalinic chalcones isolated, or in association with standard OSCC chemotherapy drugs, in human OSCC cells and normal keratinocytes. To evaluate the expression of inflammation- and tumor-associated genes, a microarray assay was performed. **Methods**: After reaching confluence, the human OSCC cell line HN30 and normal keratinocytes HaCat cells were seeded at $5x10^3$ cells/well and $8x10^3$ cells/well in DMEM/10% FBS, in 96-well plates, respectively. They were exposed for 48 h to two different quinoxaline-based chalcones, namely N17 and N23 (both 2.5 µg/ml) alone or in combination with subthreshold concentrations of the chemotherapy compounds 5-fluorouracil (1 µg/ml), cisplatin (3 µg/ml) and docetaxel (0.012 µg/ml). Cell viability was evaluated by MTT assay. To assess the long-term effects of treatments, HN30 cells were seeded at $1x10^4$ cells/well, in 24-well plates, and allowed to grow for 24 h. The medium was changed prior to treatments, and the cells were counted over 20 days. For microarray assay, the HN30 cells were seeded at $1x10^5$ cell/well, in 6-well plated, and exposed to chalcones N17 and N23 (5 mg/ml) during 48 h. **Results**: At 48 h, cisplatin alone was able to reduce the viability of HN30 and HaCat, by 52 ± 7% and 60 ± 0.2%, respectively. The combination of cisplatin with chalcones N17, N23, 5-fluorouracil, and 5-fluorouracil plus docetaxel, displayed inhibitions of 66 ± 2%, 63 ± 4%, 69 ± 1% and 63 ± 4% on HN30 cell line, and 65 ± 3%, 58 ± 2%, 68 ± 1% and 73 ± 1% on HaCat cells, correspondingly. Chalcones N17 and N23 (5 mg/ml) caused a down-regulation of inflammation and tumor-related genes, namely REL, ATP5A1, STAT1, CASP9, ACTB and GPD2. In long-term experiments, the HN30 cell line was highly sensitive to the combination of cisplatin with both chalcones. **Conclusions**: The combination of quinoxaline-based chalcones with standard chemotherapy agents can be a useful alternative for OSCC management, with less side effects when compared to standard treatments. Further studies are in progress in order to gain further insights into the mechanisms of action of the selected compounds. **Financial Support**: FINEP/PUCRSINFRA #01.11.0014-00; CNPq, CAPES, PUCRS.

10.006 Assessment of *in vitro* effects of the quinoxaline-derived chalcone N9 in breast cancer cells. Erig TC[1], Mielcke TR[2,3], Mascarello A[4], Chiaradia LD[4], Nunes RJ[4], Campos MM[2,3,5] [1]PUCRS – Pharmacy, [2]PUCRS – Toxicology and Pharmacology, [3]PUCRS – Medicine and Health Sciences, [4]UFSC – Chemistry, [5]PUCRS – Dentistry

Introduction: Breast cancer is highly prevalent in women worldwide, and its incidence has been increasing during the last years. The prognosis and treatment are based on clinical and histological findings, taking into consideration the positive expression of hormone receptors (estrogen and progesterone) and the immunopositivity for human epidermal growth factor receptor 2 (HER2). Indeed, HER2-positive, as well as triple negative tumors (negative for hormone receptors and HER2) tend to be more aggressive, presenting a poorer prognosis (Lam et al., Cancer Treat Rev. 40: 129, 2014). It has been suggested that chalcones might represent potential alternatives to treat many types of cancer, including breast cancer (Karthikeyana et al., Recent Pat Anticancer Drug Discov. 10: 97, 2015). **Aims**: This study was designed to evaluate the *in vitro* effects of the quinoxaline-derived chalcone, namely N9, on breast cancer cell lineages with different malignancy grades. Attempts have also been made to evaluate the effects of the combination of N9 with classical chemotherapeutic drugs. **Methods**: The breast cancer cell lines MDA-MB-231 (triple negative) and SKBR-3 (HER2-positive) were seeded at 8x10³ cells/well and 6x10³ cells/well in RPMI/10% FBS, in 96-well plates, respectively. They were exposed to N9 (0.5 to 10 µg/ml), for 24 h, 48 h and 72 h. In separate experiments, N9 (2.5 µg/ml) was tested in combination with subliminal concentrations of the chemotherapy compounds cisplatin (2 and 7.5 µg/ml), 5-fluorouracil (5-FU, 6.5 □g/ml) and cyclophosphamide (CYP, 1390 µg/ml), for an incubation period of 48 h. Control experiments were carried out with the addition of DMSO (vehicle control, 0.01%). The MTT assay was used to assess the percentage of viability. **Results**: The chalcone N9 produced concentration- and time-dependent inhibition of the viability of both tested cell lines, MDA-MB-231 and SKBR-3. The maximal inhibitions were obtained at the concentration of 2.5 □g/ml, at 72 h, corresponding to 72 ± 1% and 77 ± 4%, for MDA-MB-231 and SKBR-3, respectively. For MDA-MB-231 cells, the chalcone N9 (2.5 □g/ml) produced an inhibitory rate of 56 ± 6 %, whereas cisplatin (7.5 µg/ml), 5-FU (6.5 □g/ml) and CYP (1390 µg/ml) produced inhibitions of 49 ± 5 %, 30 ± 11 % and 22 ± 13 %, at 48 h. In the combination protocols, the percentages of inhibition were 74 ± 3 %, 67 ± 8 % and 68 ± 6 %, for N9+cisplatin, N9+5-FU and N9+CYP, respectively. For SKBR-3, none of the tested combinations displayed significant synergistic effects. **Conclusions**: The present data revealed marked anti-tumor effects for the quinoxaline-derived chalcone N9, in both the triple negative MDA-MB-231 and HER2-positive SKBR-3 cell lines. Noteworthy, N9 was able to interact in a synergistic manner with standard chemotherapy agents, according to assessment of MDA-MB-231 cells. It is possible to suggest that this combination might result in less adverse effects, representing a promising option to treat breast cancer in future. Additional studies are in progress to evaluate the *in vitro* effects of these combinations.

10.007 Role of endogenous glucocorticoids in diabetes-induced increase in B16F10 melanoma lung metastases. Araújo AF[1], Carvalho VF[2], Diaz BL[1] [1]UFRJ, [2]Fiocruz

Introduction: Diabetes complications are a product of a hormonal imbalance that includes deficiency in insulin production and relative excess of hyperglycemic hormones such as glucocorticoids. Such complications include higher incidence and worse prognosis of cancer. In this study, our aim was to investigate the impact of uncontrolled diabetes on a model of melanoma hematogenous metastasis and lung colonization. **Methods**: Diabetes was induced by administering a single intravenous (i.v) injection of alloxan monohydrate (65 mg/kg), diluted with saline (0.9% NaCl), into 16-h-fasted C57Bl6 mice supplied with water *ad libitum*. For the lung metastasis model, 2 x 10^5 B16F10 melanoma cells suspended in 100 µL saline were injected in the tail vein 14 days after diabetes induction. To further explore noninvasively and in time-dependent fashion the metastatic growth, B16F10 melanoma cells with the firefly luciferase transgene (B16F10-Luc), B16F10-Luc cells were injected and luminescence was quantified after luciferin injection in an *in vivo* imaging system (IVIS). To determine the role of endogenous glucocorticoids, animals were treated with steroid receptor antagonist RU 486 (20 mg/kg in 0.5% methylcellulose (v/v in water) or vehicle alone orally once a day for 25 consecutive days, starting 3 days after diabetes induction. Data was statistically analyzed by one-way ANOVA and Bonferroni post-test ($p<0.05$). **Results**: The number of melanoma lung metastatic nodules was markedly increased in diabetic mice when compared to normoglycemic controls 14 days after i.v. inoculation of B16F10 cells (control 7.429 ± 1.587 *vs* diabetic 177.8 ± 28.58 nodules/lung, $P<0.0001$). This difference in metastatic potential was already apparent immediately after melanoma cell inoculation, as B16F10-Luc cells accumulated in greater number 2 h after i.v. injection in diabetic mice (control: 31,830 ± 11,710 *vs* diabetic: 347,300 ± 244,700 radiance units, $p<0.05$. However, the localized growth of subcutaneous B16F10 melanoma tumour weight was no different between diabetic and normoglicemic mice. Pre-treatment with RU 486 significantly reduced lung metastases in diabetic mice (Diabetic: 177.8 ± 28.58 *vs* RU 486 71.88 ± 7.549 nodules/lung, $P<0.0001$). **Conclusion**: Diabetes increases the metastatic potential of melanoma cells in a mechanism dependent on glucocorticoids. **Financial support**: CNPq and FAPERJ.