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Redox modulation by SOD mimics in renal cancer: from etiology to progression

Modulação redox por miméticos da SOD no cancro renal: da etiologia à progressão

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Abstract

The incidence of renal cancer has been increasing over the last decades along with the interest in the research and development of anticancer drugs. Cancer is a multistep process and oxidative stress has been pointed out to have a critical role on its initiation, promotion and progression. The current project addresses the redox modulation provided by superoxide dismutase mimics (SODm), which are catalytical polyfunctional antioxidants, in renal cell models focusing on important initiation and progression events.

In order to assess potential effects of SODm in the initiation process, ochratoxin A (OTA), a well-known mycotoxin, was used as a reference nephrotoxic agent and renal carcinogen. The effects of OTA were characterized in Vero cells, a monkey kidney cell line. Primarily, we studied the cytotoxicity and genotoxicity of OTA and then we used a fluorescence microscopy assay to assess whether OTA induces reactive oxygen species (ROS). The effect of the SODm was evaluated in terms of ROS accumulation and cell viability.

The role of SODm will be further studied in terms of renal cancer progression. Proliferation, adhesion, migration and invasion properties of human renal carcinoma cells will be evaluated. Overall, we intend to obtain mechanistic insights for SODm effects and discuss their importance as potential therapeutic agents for renal cancer.

Lecturer's resumé

Graduated in Pharmaceutical Sciences (FFUL-UL), PhD student in Health Sciences – Pharmacy (Univ. Alcalá / ULHT-ECTS) and Lecturer at ULHT-ECTS. He is author and co-author of some publications and communications in national and international peer-reviewed journals and meetings. He received an international award. His main research interests focus on cancer and oxidative stress areas.

Role of the Notch pathway in female reproductive function

Papel da via Notch na função reprodutiva feminina

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Abstract

The objective of this study is to evaluate the association between Notch signaling, genital cellular remodeling and reproductive function, in the mouse model. We evaluated transcription and expression patterns of Notch component and effector genes in the ovary, oviduct and uterus along the estrous cycle.

Results indicate that Notch signaling is active and associated to female genital cellular remodeling, prompting for a regulatory role in ovarian follicle and corpus luteum development, and oviduct and uterus epithelial cell turnover and function. Notch signaling is operating in the ovarian cellular interstitium, and in luminal and glandular epithelia of genital tract, probably regulating intercellular communication. Part of this work was previously published:

Dynamics of Notch signaling in the mouse oviduct and uterus during the oestrous cycle, Reproduction, Fertility and Development <http://dx.doi.org/10.1071/RD15029>
Differential expression of Notch component and effector genes during ovarian follicle and corpus luteum development during the oestrous cycle, Reproduction, Fertility and Development <http://dx.doi.org/10.1071/RD13399>

Lecturer's resumé

October-2009 - Faculty of Veterinary Medicine – Technical University of Lisbon, Portugal. Master's degree in Veterinary Medicine, with the thesis in Canine Dilated Cardiomyopathy, under the supervision of Professor José Paulo Sales Luís
March 2014 - Faculty of Veterinary Medicine, University of Lisbon, Portugal. PhD in Veterinary Sciences specialty of Biologic and Biomedical Sciences, PhD thesis addressed to the Notch signaling enrolment in the reproductive function in the mouse model – under supervision of Professor Luís Lopes-da-Costa and António Duarte.
Since 2014 - Assistant Professor at the Faculty of Veterinary Medicine - Universidade Lusófona de Humanidades e Tecnologia – Disciplines of Reproduction and Physiology.

Main research:

April 2015 - Murta, D., Batista, M., Trindade, A., Silva, E., Henrique, D., Duarte, A., Lopes da Costa, L. (2015). Dynamics of Notch signalling in the mouse oviduct and uterus during the oestrous cycle. Reproduction, Fertility and Development. DOI: 10.1071/RD15029

Nov. 2014 - Murta, D., Batista, M., Trindade, A., Silva, E., Henrique, D., Duarte, A., Lopes da Costa, L. (2014). In vivo notch signaling blockade induces abnormal spermatogenesis in the mouse. Plos One, 9 (11): e113365. Doi: 10.1371/journal.pone.0113365

April 2014 - Murta, D., Batista, M., Silva, E., Trindade, A., Mateus, L., Duarte, A., Lopes da Costa, L. (2014). Differential expression of Notch component and effector genes during ovarian follicle and corpus luteum development during the oestrous cycle. Reproduction, Fertility and Development. DOI: 10.1071/RD13399

August 2013 - Murta, D., Batista, M., Silva, E., Trindade, A., Henrique, D., Duarte, A., Lopes da Costa, L. (2013). Dynamics of Notch pathway expression during mouse testis post-natal development and along the spermatogenic cycle. Plos One, 8 (8). DOI: 10.1371/journal.pone.0072767

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Skin permeation and *in vivo* antioxidant activity of gelatin nanoparticles

Permeação cutânea e atividade antioxidante in vivo de nanopartículas de gelatina

Camila Areias de Oliveira

Universidade de São Paulo, Brasil

Isolation, modeling and phytosome forms of antiproliferative compounds from *Plectranthus* spp

Isolamento, modelação e formulações fitossomais de compostos antiproliferativos obtidos de plantas do género Plectranthus

Diogo Matias^{1,3}, Marisa Nicolai¹, A. S. Fernandes^{1,2}, Nuno Saraiva¹, M. Fátima Simões¹, Ana Diaz Lanza³, Catarina Pinto Reis¹, Patricia Rijó^{1,2}

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Abstract

The purpose of this study was the assessment of the skin permeation and *in vivo* antioxidant activity of gelatin nanoparticles incorporated in sunscreens formulations. The *in vitro* permeation study indicated that rutin-loaded nanoparticles do not present the tendency to permeate the skin. After 24 hours, 1.1 ± 0.09 mg (recovery – $106.0 \pm 8.5\%$) of the flavonoid was quantified in the donor compartment. Additionally, no amount of rutin was quantified with precision and accuracy in the stratum corneum, the remaining full thickness skin or the receptor compartment. The *in vivo* antioxidant activity results indicated that the presence of gelatin nanoparticles (GNPs) decreased the intensity of the erythema caused by ethyl nicotinate, as noted in the slope of the tangent line in the hyperemia parameter. However, a statistically significant difference was not achieved in the area under the curve parameter. In conclusion, these assays characterized the nanomaterials as an innovative platform for bioactive sunscreens.

Lecturer's resumé

Camila Areias de Oliveira is a pharmacist that has been working in the cosmetic field for over 6 years. Nowadays she is a PhD student at Universidade de São Paulo and works in research and development of cosmetic products, with emphasis on developing bioactive sunscreens.

Abstract

The natural products have been a valuable source of new bioactive lead molecules which could become or inspire new drug entities. Previous studies with plants from genus *Plectranthus* (Lamiaceae) yielded abietane diterpenes with interesting cytotoxic activities. In this study, extracts of three plants of this genus were screened for their cytotoxic activities. The *P. madagascariensis* extract, obtained from maceration with acetone showed the most promising cytotoxic activity with an IC₅₀ of 64 µg/mL in MDA-MB-231 line cancer cells. The main compounds were identified, using HPLC-DAD, as rosmarinic acid, 6α-7β-dihydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and coleon U. The isolated compounds were individually tested, using the same model, and coleon U was the most cytotoxic with an IC₅₀ of 16.25 µg/mL in same cell culture line. In addition, the fractionation of this extract, using chromatographic techniques yielded a new compound, 7α-formyloxy-6β-hydroxyroyleanone (identified mainly by spectroscopic techniques 1D and 2D-NMR). The most cytotoxic extract was formulated into a phytophospholipid complex being the conditions optimized. Acetone (the selected solvent) and reaction time of 2h produced the smallest particles with mean diameter of 96.2 ± 11.2 nm. Those particles were then microencapsulated into chitosan particles (mean size of 3402.1 ± 439.0 nm) and finally coated with albumin (mean size 33.1 ± 3.6 µm) in order to achieve gastric resistance.

Lecturer's resumé

Diogo Matias is pharmacist (MPharm) and PhD student in the Health Sciences Doctoral program at the Universidade Lusófona de Humanidades e Tecnologias (Lisbon, Portugal) in collaboration with the Alcalá University (Madrid, Spain).

His main research interests are natural products isolation, identification and the screening of biological activities, namely antimicrobial, antioxidant and cytotoxic. The development of new drug delivery systems for natural products is also a research area of interest.

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Role of SOD mimetics in breast cancer metastization

Papel de miméticos da SOD em metastização do cancro da mama

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Abstract

Breast cancer metastases are a major cause of cancer mortality. Reactive species (RS) have been greatly implicated in cell migration and invasion, key determinants for the metastatic process. Superoxide dismutase mimetics (SODm) are catalytical polyfunctional antioxidants that mimic superoxide dismutases and interact with different RS. SODm could impact tumor proliferation and metastases formation in breast cancer.

In this work we evaluated the role of two SODm, alone or in combination with doxorubicin (dox) in the viability, migration/invasion and intracellular RS levels in two human breast cancer cell lines: MCF7 (low aggressiveness) and MDA-MB-231 (highly aggressive).

Primarily, the effect of the two SODm was evaluated in terms of cell viability in both cell lines (MTT assay). Cell migration was firstly evaluated by the wound healing assay in both cell lines exposed to the two SODm alone or in combination with dox. Chemotaxis and chemoinvasion were evaluated by the transwell inserts assay and intracellular RS levels were assessed by fluorescence microscopy using dihydroethidium (DHE) and dihydrorhodamine 123 (DHR) probes.

This work shows that redox regulators can modulate cell migration and invasion. Our data suggest that the two SODm, in combination with dox, may be beneficial in breast cancer treatment, by interacting at different levels. Work is in progress to further explore the mechanisms responsible for the effects observed, namely in terms of matrix metalloproteinases (MMPs) activity, which are important enzymes for the invasion mechanisms.

Lecturer's resumé

Ana Flório is graduated in Chemical Engineering (Biotechnology field) by Technical University of Lisbon, Portugal in 2001 and MSc in Biotechnology – Biochemical Engineering by Technical University of Lisbon, Portugal in 2008. Presently she is a PhD student in Pharmacy (Toxicology speciality) at the Faculty of Pharmacy, University of Lisbon. Since 2009, she is equivalent to adjunct professor at Estoril Higher Institute for Tourism and Hotel Studies (ESHTe) in Estoril, Portugal, teaching Food Chemistry I and Food Chemistry II to first year students of Cookery and Food Production. Ana Flório is author and co-author of some publications and communications in national and international peer-reviewed journals and meetings. Her main research interests focus on breast cancer and oxidative stress/redox modulation areas.

Royleanone derivatives from *Plectranthus grandidentatus* and its topical application through drug delivery nanosystems

*Derivados de royleanona de *Plectranthus grandidentatus* e a sua aplicação tópica em nanossistemas para veiculação de fármacos*

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Abstract

A large number of phytochemical studies have been carried out in *Plectranthus* species due to the presence of metabolites with antimicrobial activity. 7 α -acetoxy-6 β -hydroxyroyleanone is an abietane diterpene isolated in significant amounts from *Plectranthus grandidentatus*. This compound has strong antibacterial activity against Gram-positive strains, especially against resistant *Staphylococcus aureus* strains (MRSA with MIC values between 3.12 to 15.63 μ g.mL⁻¹). In the present work, the 7 α -acetoxy-6 β -hydroxyroyleanone extraction from *P. grandidentatus* was optimized using different extraction methods (decoction, infusion, microwave and ultrasonic irradiation) and CO₂ supercritical fluid (SCF) extraction and then characterized through HPLC-DAD. The highest yield was obtained for the SCF extract (57.35 μ g.mg⁻¹ of extract). The mechanism of action of this bioactive compound was studied in a bacterial strain MRSA /VISA (CIP 106760) by the growth curve analysis, the cell leakage assay, the SEM morphology analysis, the influence in surface charge and the lytic capacity of the bacteria when exposed to different compound concentrations (2 $\times$ MIC, MIC and MIC/2). The reactivity and derivatization of the antimicrobial royleanone are now under study to improve its bioactivity, using the Mitsunobu reaction. The resultant chemical structures of the isolated derivatives will be confirmed by spectroscopic methods. The antimicrobial activity of all derivatives will be also evaluated. Most active compounds will be conjugated with silver nanoparticles for treatment of topical infections. In conclusion, the mechanism of action studies are important to unveil the mode of action of 7 α -acetoxy-6 β -hydroxyroyleanone against a resistant bacteria strain. The new derivatives obtained by hemi-synthesis are potentially proposed to increase its bioavailability and bioactivity.

Lecturer's resumé

Filipe Pereira have a master degree in medicinal chemistry and therapeutics at the Faculdade de Farmácia (U. Lisboa) and is a PhD student in the Health Sciences Doctoral program at the Universidade Lusófona de Humanidades e Tecnologias (Lisbon, Portugal) in collaboration with the Alcalá University (Madrid, Spain). His latest work is related to isolation and hemi-synthesis of natural compounds with antibacterial activity.

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Research in dental veterinary medicine

*Investigação em medicina dentária veterinária***João Filipe Requiça^{1,2,3}**¹Faculdade de Medicina Veterinária, Universidade Lusófona, Lisboa, Portugal²Unidade de investigação 3B's, Universidade do Minho, Guimarães, Portugal³CBiOs - Research center for Biosciences and Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal

Abstract

A periodontite é uma doença inflamatória que afeta o periodonto o qual é o órgão que envolve e sustenta o dente. Quando não tratada, esta conduz à esfoliação dentária devido à destruição do osso alveolar e do ligamento periodontal, e coloca em risco a vida do doente devido à dispersão sanguínea de mediadores inflamatórios e microrganismos patogénicos.

A reconstrução desses defeitos, tanto em animais de companhia como em humanos é um verdadeiro desafio. A Engenharia de Tecidos surgiu com abordagens alternativas e promissoras envolvendo a utilização de biomateriais de suporte e de células indiferenciadas que visam induzir a regeneração do tecido alvo.

Num trabalho, recentemente desenvolvido, obteve-se uma matriz biodegradável à base de uma mistura de amido e policaprolactona e combinada com células estaminais derivadas do tecido adiposo (ASCs) canino. Este scaffold foi proposto para utilização em regeneração periodontal dado que tem o potencial de guiar o crescimento dos diferentes tecidos periodontais e de ser osteocondutor.

Atualmente, encontramos-nos a caracterizar as ASCs caninas bem como de outras espécies com interesse em Medicina Veterinária, de forma a abrir portas para o desenvolvimento de novas terapias celulares.

Considerando que a doença periodontal tem uma elevada base genética, realizamos também trabalhos de identificação de polimorfismos de nucleótido simples associados ao aparecimento da doença em cão. Este estudo poderá, num futuro próximo, contribuir para o desenvolvimento de testes que avaliem a susceptibilidade a esta patologia.

A cirurgia reconstrutiva dos defeitos periodontais e ósseos está associada a uma elevada dificuldade técnica e que é inerente às características anatómicas da cabeça e da cavidade oral. A tecnologia de prototipagem rápida tem revelado um enorme interesse em Medicina Humana como meio auxiliar no planeamento cirúrgico. O intuito deste projeto é o de otimizar a tecnologia de impressão 3D e aplicá-la na área da cirurgia maxilofacial veterinária.

A investigação na área da doença periodontal, tanto em cão como em outros animais de companhia, é essencial para a melhoria dos métodos de diagnóstico, da vez mais precoces, e das suas técnicas terapêuticas.

Lecturer's resumé

Licenciado em Medicina Veterinária na Universidade de Trás-os-Montes e Alto Douro, em 2007. Mestre em Medicina Veterinária na área das neoplasias da cavidade oral (2011). Especialista Universitário em Medicina Dentária e Cirurgia Maxilofacial na Universidade Complutense de Madrid, desde 2011.

Doutoramento em Ciências Veterinárias pela UTAD e em colaboração com a Unidade de Investigação 3B's da Universidade do Minho e com a Universidade de Santiago de Compostela (2013). Certificado de Investigador-Coordenador da DGAV. Professor Auxiliar da Faculdade de Medicina Veterinária da Universidade Lusófona, desde 2013. Membro da Unidade de Investigação 3B's, pertencente ao Laboratório Associado ICVS - Instituto para a melhoria da Vida e Saúde/3B's. Colaborador do grupo CBiOs da U.Lusófona.

Marek's disease virus (MDV) ubiquitin-specific protease (USP) performs critical functions beyond its enzymatic activity during virus replication

*O papel das proteases específicas da ubiquitina na replicação viral e oncogénese induzida pelo vírus da doença de Marek***Inês M.V. Veiga¹, Keith W. Jarosinski², Benedikt B. Kaufer³, Nikolaus Osterrieder³**¹CBiOs - Research center for Biosciences and Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisboa, Portugal²University of Iowa Carver College of Medicine, 375 Newton Rd, Iowa City, IA 52242, USA³Institute für Virologie, Freie Universität Berlin, Philippstraße 13, 10115 Berlin, Germany

Abstract

Marek's disease virus (MDV) encodes an ubiquitin-specific protease (USP) at the N-terminus of its UL36 gene, which is highly conserved among herpesviruses and is important for MDV replication and pathogenesis in the chicken, MDV's natural host. In order to understand the role of this enzyme, several MDV mutants were generated via two-step red mediated recombination and their in vitro phenotypes were evaluated using plaque size and growth kinetics assays. The study of the obtained phenotypes showed that the N-terminus of pUL36 is essential for MDV replication and cannot be complemented by ectopic expression of MDV USP. Additionally, it was found that the region located between two conserved residues comprising the active site cysteine (C98) is also essential for MDV replication. Based on these observations, it was concluded that MDV USP contributes to the structure of UL36-encoded protein and affects MDV replication and oncogenesis beyond its enzymatic activity.

Lecturer's resumé

Inês Margarida Berenguer Veiga has a degree in veterinary medicine (DVM) from the Lisbon Technical University (2007) and a PhD in Biomedical Sciences (2013) from the Freie Universität (FU) Berlin. The aim of her PhD project was to uncover the role of the UL36-encoded ubiquitin-specific protease (USP) in Marek's disease virus (MDV) replication and tumorigenesis and was performed under Professor Nikolaus Osterrieder supervision at the Institute of Virology from the Department of Veterinary Medicine. She has been working as a lecturer at the Faculty of Veterinary Medicine from the Lusófona University in Lisbon since October 2013. Around the same time she was also granted the one-year Doherty Research School (DRS) Honors 2013 fellowship to perform a research project at the FU Berlin Institute of Poultry Diseases, whose aim was to identify the presence of Ornithobacterium rhinotracheale in both wild and domestic Portuguese bird samples.