MICRO BIOTEC'11

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was no apparent relationship between the geographic origin of the samples and the MATR allelic profiles of MAH strains, corroborating the results of the previous typing study using IS*1245*-RFLP [1]. MATR-VNTR typing revealed to be simple, fast and affordable, having a high level of reproducibility and discriminatory power for MAH.

[1] Domingos et al. *2008. Vet. Rec. 163: PO12161-1-9. [2] Inagaki* et al. *2009. J. Clin. Microbiol. 47: 2156-2164.Célia Leão is a recipient of a PhD grant from the Portuguese "Fundação para a Ciência e a Tecnologia" (SFRH/BD/62469/2009)*

PS3: 39

HepG2 cells under starvation-induced autophagy are susceptible to cell death caused by oleanolic acid but not by ursolic acid

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Cancer incidence is increasing worldwide mainly due to changes in diet, life style and increased lifespan. In particular, liver cancer is the fifth most common cancer in the world and the third most common cause of cancer mortality. Plant phytochemicals are a good and promising source of anticancer compounds. In a previous work, we reported the potential of ursolic acid (UA) to induce cell death and to inhibit proliferation in colorectal cancer cells [1]. This natural triterpenoid UA was also shown to activate JNK and to modulate molecular markers of autophagy (Xavier *et al.*, unpublished data). In the present study, we tested the ability of two isomer triterpenoids, UA and oleanolic acid (OA), to induce cell death and modulate autophagy in human hepatocellular carcinoma cell line (HepG2 cells). For that, the effect of these phytochemicals on cell death was evaluated by MTT assay and propidium iodide staining, in complete and starvation medium. Autophagy markers were evaluated by western blot and fluorescent microscopy. Contrary to our previous data with other cell lines, HepG2 cells were less susceptible to UA and, unexpectedly, OA was a more potent inducer of cell death than UA. Interestingly, starvation-induced autophagy sensitized HepG2 cells to cell death caused by OA, but not by UA. The IC_{50} of OA decreased from 50 μM in complete medium to 3.5 μM in starvation medium. Although UA and OA increase the levels of autophagy markers LC3-II and p62, as well as the number of acidic vacuoles (as assessed by MDC staining), the cell death induced by OA was not prevented by inhibitors of autophagy and of lysosome proteases. Overall, the results seem to indicate that autophagy is not involved in cell death induced by OA. Interestingly, methyl-β-cyclodextrin (a polymer able to decrease membrane cholesterol content) prevented OA-induced cell death. In conclusion, these results seem to indicate that cellular membrane biophysics are affected by OA, in particular during starvation and with involvement of cholesterol, which leads to sudden cell death. In the future OA can be viewed as specific drug for cancer treatment in particular cell physiological conditions.

[1] Xavier *et al., Cancer Letters,* 2009, 281: 162-70. *Acknowledgements:* This work is supported by FCT research grant NaturAge – PTDC/QUI-BIQ/101392/2008, which is co-funded by the program COMPETE from QREN with co-participation from the European Community fund FEDER.

PS3: 40

Human leptospirosis in Azores: from past to present

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Leptospirosis is probably the most widespread and frequent zoonotic disease in the world. Since 1993, Azores islands (Portugal) have been considered endemic for this worldwide zoonosis. High precipitation and moderate temperature, together with high densities of rodents (the primary reservoirs of leptospires), provide an extensive dissemination of these spirochetes allowing their survival in nature.The high prevalence and mortality rates by Leptospirosis, the growing number of detected human cases and the lack of local laboratory facilities for diagnosis, contributed to the implementation and development of a research project entitled "*Epidemiology and Control of Leptospirosis in Azores*" under USA Scientific-Cooperative Agreement No. 58-4001-3-F185. The project, undertaken between 2004 and 2008, was founded on four research areas: *i)* human epidemiology; *ii)* reservoir's ecology; *iii)* laboratory diagnosis, including the technology transfer from Leptospirosis Reference Laboratory (LRL) at IHMT, to local hospitals on São Miguel and Terceira islands; and *iv)* evaluation of the knowledge attitudes and practices of the population facing exposure to *Leptospira* infection.The purpose of this study was to evaluate the current trend of the Leptospirosis in Azores, based on human positive cases registered on LRL databases concerning the timing, before, during and after project completion.A total of 1182 sera, received at LRL (IHMT), between 1993 and 2010, from Azorean patients with Leptospirosis clinical suspicion, were analyzed during the referred period, by the reference Microscopic Agglutination Test (MAT) in order to