

## BOLSA DE INVESTIGAÇÃO (M/F)

**Referência:** PTDC/SAU-FCF/101177/2008

**Título do Projecto:** “A role for Nrf2/ARE cytoprotective Signalling in iron overload. Adaptive response to oxidative stress as a disease modifier in HFE-associated hereditary hemochromatosis.”

**Código interno:** PR250808

Está aberto concurso para recrutamento de um(a) bolseiro(a) de Investigação para colaborar no projecto acima referido, financiado pelo programa COMPETE - Programa Operacional Factores de Competitividade na sua componente FEDER e pelo orçamento da Fundação para a Ciência e a Tecnologia na sua componente OE.

A bolsa, em regime de exclusividade, terá a duração de 1 ano, eventualmente renovável, com início previsto em 1 de Agosto de 2010.

O valor mensal da bolsa será de € 980,00, pago por transferência bancária (preferencialmente).

**Local de trabalho:** IBMC - Instituto de Biologia Molecular e Celular (Grupo IRIS - Iron Genes and Immune System), Porto, Portugal.

**Programa de trabalho:** ver anexo.

### **Perfil pretendido:**

Os candidatos devem ter o grau de mestre na área de Ciências Biológicas, Biomédicas, Farmacêuticas ou afins, sendo dada preferência a quem tiver média de licenciatura igual ou superior a 14 valores e manifesta experiência com técnicas necessárias à boa execução do referido projecto.

O prazo para recepção de candidaturas decorre de 23 de Junho a 7 de Julho de 2010.

As propostas deverão incluir carta de motivação, CV e duas cartas de recomendação e ser enviadas por correio electrónico para [candidaturas@ibmc.up.pt](mailto:candidaturas@ibmc.up.pt) com indicação do código interno (PR250808).

A contratação será regida pelo estipulado na legislação em vigor relativamente ao Estatuto de Bolseiro de Investigação Científica, nomeadamente a Lei 40/2004, de 18 Agosto, e o Regulamento de Bolsas de Investigação Científica do IBMC ([www.ibmc.up.pt/fellowships.php](http://www.ibmc.up.pt/fellowships.php)).

“A role for Nrf2/ARE cytoprotective signalling in iron overload. Adaptive response to oxidative stress as a disease modifier in HFE-associated hereditary hemochromatosis”

**Supervisor:** Tiago L. Duarte

**Project Summary:**

**BACKGROUND**

HFE-associated hereditary hemochromatosis (HH) is the most common genetic disorder of iron overload among Caucasians. If untreated, it can lead to total body iron overload with secondary tissue damage in several organs attributed to oxidative stress. Most HH patients are homozygous for the C282Y mutation in the HFE gene. However, symptoms are highly variable, with most C282Y homozygotes in non-clinical populations not exhibiting any iron overload-related disease, despite the elevated iron levels. The low penetrance of the C282Y mutation indicates that C282Y homozygosity is a necessary but not sufficient factor in causing the disease. It remains elusive why some patients exhibit a clinically severe disease while most C282Y homozygotes are apparently healthy.

There is evidence that human cells expressing C282Y HFE are more resistant to oxidative stress than cells expressing wt HFE but the mechanism is not understood. The C282Y HFE mutant protein is retained in the endoplasmic reticulum (ER), causing ER stress and the onset of an unfolded protein response (UPR). Moreover, the C282Y HFE-induced protection from oxidative stress is associated with increased expression of the ER chaperone calreticulin. This suggested that differences in the magnitude of the UPR elicited by C282Y HFE would influence the individual capacity to respond to the iron overload-associated oxidative stress. Further evidence for an adaptive response came from the observation that C282Y/C282Y lymphocytes are more resistant to diepoxybutane-induced chromosome breakage than lymphocytes from controls or patients with secondary forms of iron overload. Systemic adaptation to oxidative stress is associated with the induction of a set of genes encoding proteins with antioxidative and cytoprotective functions, many of which contain antioxidant response elements (AREs) in promoter regions. Transcription factor Nrf2 regulates transcriptional induction of ARE-containing genes and promotes cell survival in response to oxidative stress. There is growing evidence that Nrf2 is an important modifier of diseases involving oxidative stress. Moreover, polymorphisms in the Nrf2/ARE pathway may compromise the adaptive antioxidant response. Importantly for this proposal, Nrf2 activation was recently linked to the UPR, via ER stress-associated production of reactive oxygen species and/or PERK-dependent phosphorylation. Whilst the protective role of Nrf2 in iron overload disease remains undetermined, we hypothesize that resistance to oxidative stress may be a modifier of disease progression in HFE-HH and propose to investigate whether this is attributed to activation of Nrf2/ARE signalling via the UPR.

## AIMS and METHODS

The first aim of this project will be to determine the role of Nrf2 antioxidant/cytoprotective signaling pathway in cellular defense against iron toxicity. To this end, we will employ several cellular models of iron overload. Secondly, we will determine the involvement of Nrf2 and target cytoprotective genes in the protection of cells transiently/stably transfected with C282Y HFE from oxidative stress. The requirement for Nrf2 will be tested by gene silencing. The above findings will then be confirmed in clinical samples, by assessing the activation of Nrf2 and expression of Nrf2-regulated genes in peripheral blood cells of C282Y homozygous HH patients, and correlating them with markers of UPR and clinical manifestations of iron overload. Finally, experiments are planned to investigate whether the Nrf2 signalling pathway can be pharmacologically induced in order to increase resistance to iron overload-associated oxidative stress. This will be firstly achieved *in vitro* and subsequently *in vivo*, by supplementing the diet of HFE<sup>-/-</sup> mice, a model of spontaneous iron overload, with small-molecule inducers of Nrf2 pathway and measuring parameters of oxidative damage.

## EXPECTED RESULTS

This project will elucidate the putative protective role of Nrf2 in iron overload disease. It is also expected that this study will improve the current understanding of the systemic adaptation to oxidative stress in HFE-HH, and provide important new information that will help determine which C282Y homozygotes are at increased risk to develop iron overload-related tissue damage.