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Filipa Oliveira & Fernando Cardoso

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Interleukin gene cloning and expression in *E. coli*

Filipa Oliveira[a,b] and Fernando Cardoso[b,c]

[a]Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (FCT-UNL), Caparica, Portugal; [b]Instituto de Higiene e Medicina Tropical (IHMT-UNL), UEI Parasitologia Médica, Estrada da Junqueira,100, Lisboa, Portugal; [c]Global Health and Tropical Medicine (GHTM), Estrada da Junqueira,100, Lisboa, Portugal

ABSTRACT

Introduction: Cytokines are a large category of protins; they play various functions from inflammation response to cancer development and sepsis. Over-expression or problems in production control of this small peptides can lead to the development of various diseases, including autoimmune diseases, such as rheumatoid arthritis. Inhibition of some of these proteins can result in a therapy that can ease certain symptoms of cases of immunologic diseases.
Material and Methods: This work consists in cloning and expressing six truncated proteins of interleukins: IL-1α, IL-1β, IL-17A, MIF, TNFSF11 and CD20. These synthetic genes were synthesised with the *E. coli* codon usage and inserted in pNZY29 cloning vector (Nzytech). These genes were isolated by amplification by PCR method with specific primers, gel purified and cloned in the pLATE31 (Thermo) expression vector. The purified expression vectors were used to transform the following *E. coli* expression strains: BL21 (DE3), BL21-Gold (DE3), BL21-CondonPlus RIPL (DE3), BL21- Gold (DE3) pLysS, BL21 Star (DE3), BL21 SHuffle, BL21 SHuffle LysY and BL21 XJB (DE3) and expressed and detected according to a previous work [1].
Results: The cloning and expression system used was a directional cloning of PCR-generated fragments and a DNA ligase free method, with this system was achieved a high cloning efficiency (80–90%). The polyacrylamide gel allowed the detection of the strains with higher levels of expression of the recombinant proteins. In this study the level of protein expression was different in each *E. coli* strain tested and was dependent on the protein expressed.
Discussion and Conclusions: We succeeded to produce IL1-α IL1-β IL17A, MIF, TNFSF-11 and CD20 recombinant proteins in different *E. coli* strains but the expression is strain dependent according to the protein. These recombinant proteins are capable of functioning as antigens to produce monoclonal and recombinant antibodies and recombinant peptide ligands. Next steps in the research include culture conditions optimisation to increase recombinant protein yield and the selection and production of recombinant peptide ligands from phage libraries and it is used in an *in vitro* inhibitory and ELISA assay.

CONTACT Fernando Cardoso fcardoso@ihmt.unl.pt

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The genetic susceptibility linking preterm birth and periodontal disease – a review

Joana Couceiro[a,b], Ana Rita Grosso[b], Pedro V. Baptista[b], José J. Mendes[c], Alexandra R. Fernandes[b] and Alexandre Quintas[a,d]

[a]Laboratório de Patologia Molecular e Bioquímica Forense, Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Egas Moniz Cooperativa de Ensino Superior, Caparica, Portugal; [b]UCIBIO, Department of Life Sciences, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal; [c]Clinical Research Unity, Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Egas Moniz Cooperativa de Ensino Superior, Caparica, Portugal; [d]Laboratório de Ciências Forenses e Psicológicas, Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Egas Moniz Cooperativa de Ensino Superior, Caparica, Portugal

ABSTRACT

Introduction: Preterm birth (PTB) is a major clinical and public health challenge, being the main determinant of neonatal mortality and the second most common cause of death in children younger than 5 years old [1]. This condition is also responsible for 75% of neonatal morbidity which often extends to later life, resulting physical, psychological and economic costs [2]. Although some risk factors for PTB have already been described, its aetiology is still uncertain [3]. Several inflammatory diseases have been associated to PTB, including periodontal disease (PD), ranking the 6th position among the most prevalent diseases worldwide [4]. Despite the links that have been proposed, the relationship between PTB and PD is not fully understood. Nevertheless, both conditions were associated with a genetic predisposition and relevant variants were found in genes associated with the inflammatory system. The present study aims to shed light on the inflammatory network underlying PTB and PD occurrence.

Materials and methods: A literature review was conducted in B-on, Pubmed and Science Direct databases using the search terms: "preterm birth"; "periodontal disease"; "genetic variants" and "inflammation". Only peer reviewed papers in English, published between 2000 and 2019 were included. Studies using animal cell lines, animal experiments and in silico research were excluded. Six genes associated to PTB and PD were found. After, STRING biological database was used to predict protein-protein interaction for each of the genes found previously.

Results: Figure 1 shows the STRING networks for IL1A and MMP9, two of the most common genes which variants are associated both with PTB and PD. Afterwards, a search on the literature for the genes in the 1st sphere of the network was conducted to find any correlation with PTB and PD. In the end 90 variants in 40 different genes were identified associated to these conditions.

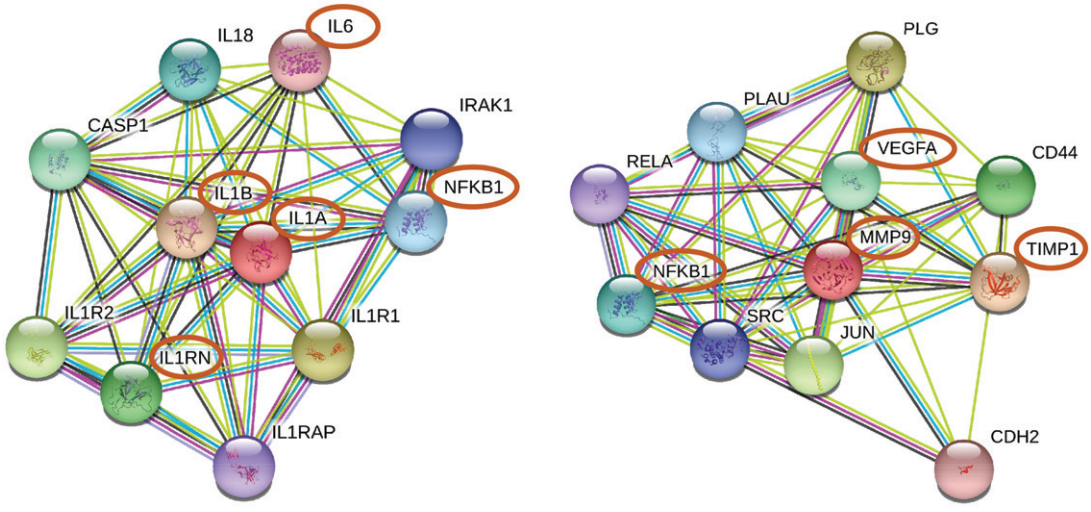

Figure 1. Example of protein networks of two key proteins involved in PTB and PD.