olink®

MOUSE EXPLORATORY

Pilot study application examples

Introduction

Using animals in research is critical to scientific understanding of the biology underlying human diseases, enabling translational medicine studies that can result in new and better therapies. Olink has developed Olink® MOUSE EXPLORATORY, the most comprehensive multiplexed protein biomarker panel designed for mouse studies. Just 1 µL of sample is required to measure 92 proteins simultaneously, and this small volume is particularly important when working with mouse models.

Several pilot studies using this mouse panel are briefly described in this document. The studies include examples in fields such as aging, cardiovascular disease, inflammation, neurology and oncology.

Study 1: Changes in protein levels in aging mice

Purpose

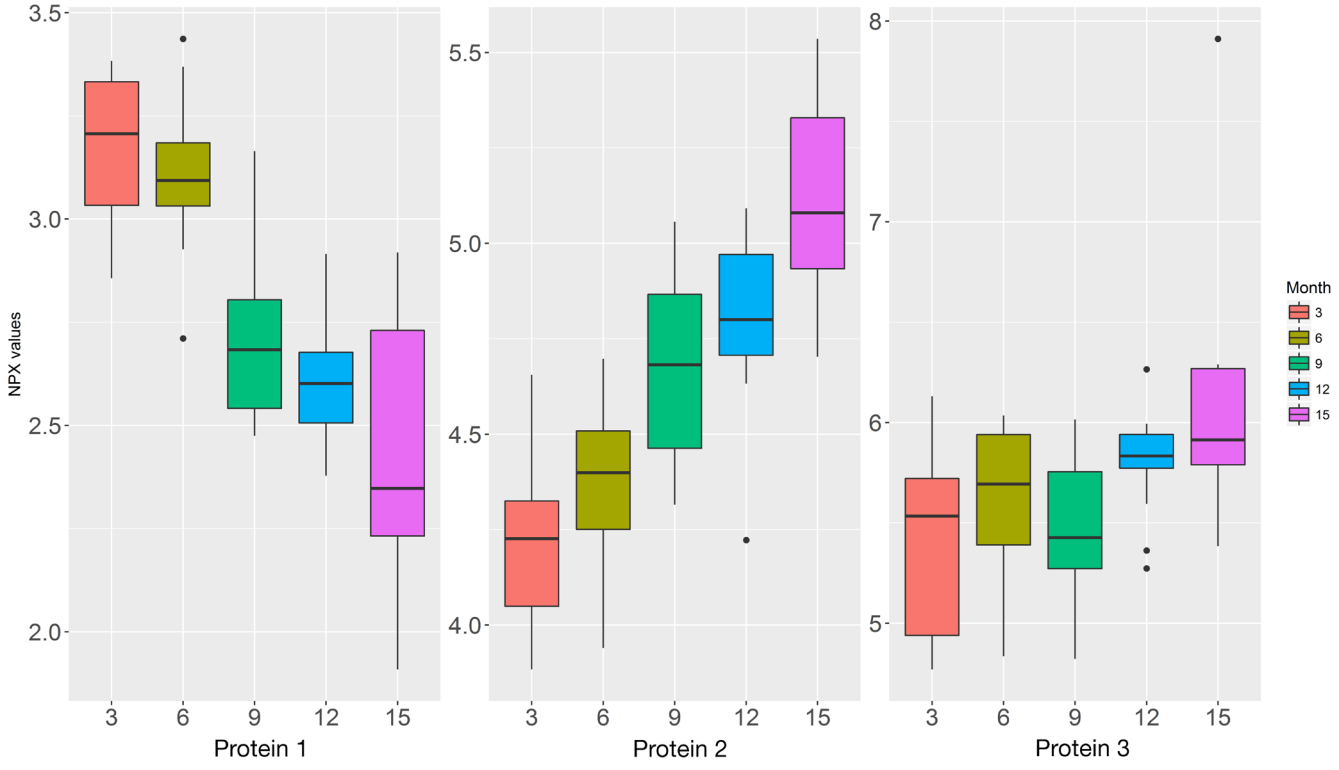
This small pilot study examined what happens in aging mice at the protein level, as well as looking at the effects of excercise. Consequently, the levels of 92 proteins were measured and evaluated in aging mice.

Method

Sixty mice with ages spanning from 3 to 15 months were classified as runners or non-runners. From these, 50 plasma samples were collected and run on the Olink mouse panel.

Result

A total of 80% of the proteins were detected in over 75% of the samples, which indicates good detectability. The mouse samples worked well with the Olink assay and generated reproducible data. A multivariate regression model revealed 16 proteins to be differentially expressed depending on the variable *Age,* and 2 proteins depending on the variable *Excercise*. There were both positive and negative associations to the investigated variables as shown in the figure below (data shown for the variable *Age* only). All protein levels are reported in Olink's Normalized Protein eXpression (NPX) units, which provides relative quantification within the assay.



Study 2: Myocardial infarction profiles in a transgenic mouse model

Purpose

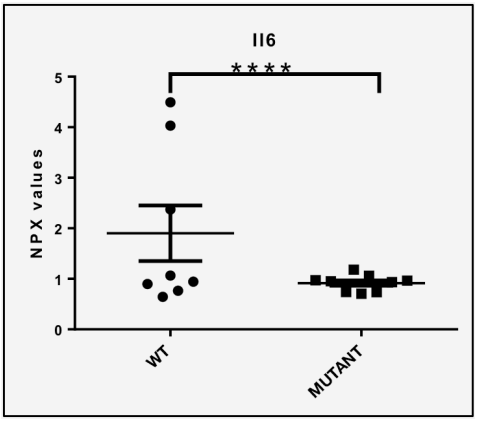
The aim of this pilot study was to identify differences in myocardial infarction protein profiles between wild-type and mutant mice. All mice had myocardial infarction but the 10 transgenic mice exhibited an additional specific cardiovascular phenotype.

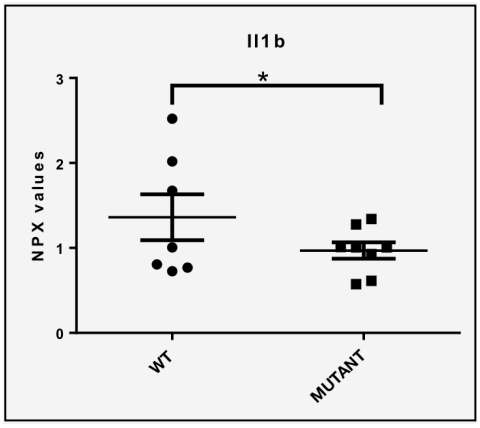
Method

Myocardial infarction was induced by ligation of the left anterior descending coronary artery in males from the transgenic mouse line and age matched wild-type mice from heterozygous breedings. The mice were 15 to 23 weeks old. At three days post infarction, the experiment was terminated and biopsies taken from the infarction areas. The biopsies were lysed and then assayed using the Olink mouse panel.

Result

Several proteins showed significant difference between wild-type and transgenic mice lysate samples, but the two most interesting were Interleukin-6 (Il6) and Interleukin-1beta (Il1b) as illustrated below.





Study 3: Allergic airway inflammation model

Purpose

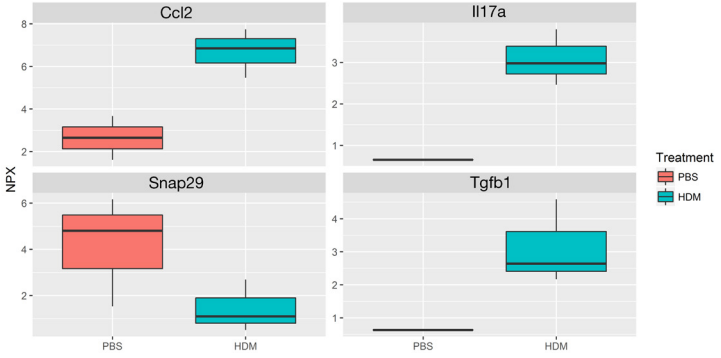
The aim of this pilot study was to detect and differentiate proteins that might be connected to allergic airway inflammation, measuring in both serum and bronchoalveolar lavage fluid (BAL).

Method

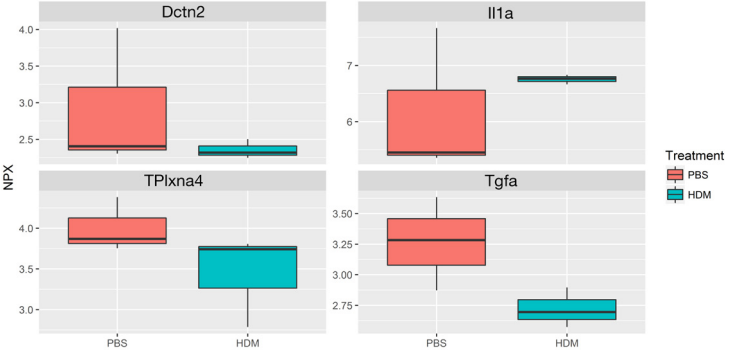
The mice were divided into two equally sized groups. In each of these, three mice were injected with house dust mites (HDM), and three mice with phosphate-buffered saline (PBS) as control. Serum and BAL samples were taken from all mice and run on Olink mouse panel. Proteins detected in more than three samples were included in the analysis.

Result

For each sample type, the four proteins that differed the most between treatments with HDM and PBS were identified. In BAL the NPX values of the proteins Ccl2, Il17a, Snap29 and Tgfb1 differed the most, as shown in the figure below.



In contrast, the proteins Dctn2, Il1a, Plxna4 and Tgfa differed the most when measured in serum, as shown in the figure below.



These preliminary results hint at possible protein bio-markers for HDM exposure, and indicate that the choice of sample type examined may be important.

Study 4: Experimental autoimmune encephalomyelitis

Purpose

In this study, the effect of ciclosporin as a treatment for a mouse model for human multiple sclerosis (MS) was examined. Experimental autoimmune encephalomyelitis (EAE) is the most commonly used experimental model for the human inflammatory demyelinating disease, MS. The aim of the study was to identify differences in protein profiles between untreated mice and mice treated with ciclosporin. The use of ciclosporin is known to lead to decreased production of inflammatory cytokines by T-cells.

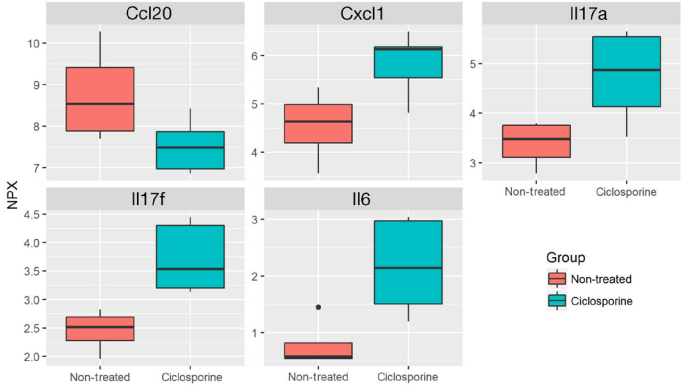
Rodent EAE has been an important model for understanding the pathogenesis and develop treatments for MS. Notwithstanding sources of variation, there are many examples of successful therapies in EAE that have also proven successful in MS. While no single model can exactly recapitulate all aspects of MS, animal models are essential in understanding the induction and pathogenesis of the disease and to develop therapeutic strategies that limit disease progression and eventually lead to effective treatments for the human disease.

Method

According to the adoptive transfer model of EAE, one set of mice was directly immunized to generate myelin-specific T-cells which were then cultured and transferred into donor mice. This study examined nine C57Bl/6 female mice 7 to 10 weeks old. Five were treated with ciclosporin and four were non-treated controls. A total of 25 serum samples were taken from the mice and assayed using the Olink mouse panel. Proteins detected in at least five samples were included in the analysis.

Result

Five proteins showed a large difference in NPX values between ciclosporine treated and non-treated mice as illustrated below. This preliminary data shows the feasibility of this approach to monitor protein expression responses to drug treatments in mouse model systems.



Study 5: PD1 inhibitor study for B16 melanoma

Purpose

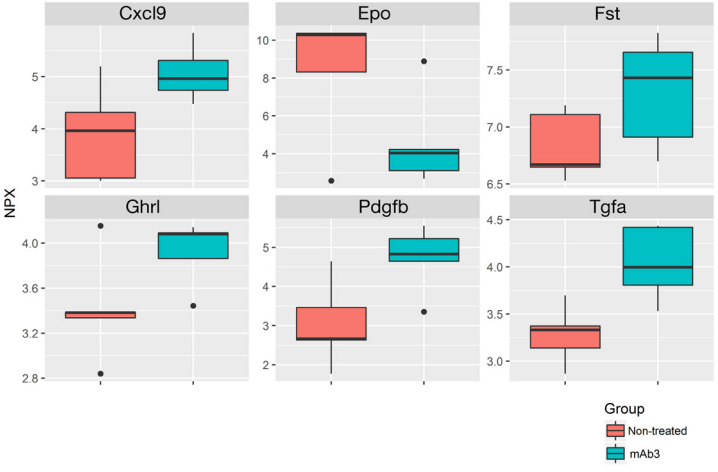
Programmed cell death protein 1 (PD1) plays an important role in down-regulating the immune system and its role in cancer immune evasion is well established. Drugs that target proteins such as PD1, known as checkpoint inhibitors, have become centrally important in the field of immuno-oncology. B16 melanoma is a murine tumor cell line used for research as a model for human skin cancers. All mice in this pilot study had B16 melanoma and the aim was to identify differences in protein profiles between untreated mice and those treated with the PD1 checkpoint inhibitor mAb3.

Method

This study examined 10 mice that all had B16 melanoma. Five were treated with the PD1 inhibitor mAb3 and five were non-treated controls. A total of 25 serum samples were taken from the mice and run on the Olink mouse panel.

Result

Six proteins showed a large difference in NPX values between mAb3 treated and non-treated mice as illustrated below. This study further supports the potential for the mouse panel to provide useful data when monitoring drug treatments in mouse models.



Conclusions

The five pilot studies carried out using Olink MOUSE EXPLORATORY show the potential for using this broad panel for protein biomarker discovery across a range of typical mouse model investigations.

Using just 1 µL sample, 92 proteins could be rapidly measured with good detectability in a range of sample matrices. While these pilots were small-scale, they demonstrate the feasibility of this approach for several key types of mouse investigations including pathophysiological studies of transgenic mouse lines and in monitoring the biological effects of drug treatments at the protein expression level. As demonstrated in the examples shown, the panel can support mouse model studies covering a range of important therapeutic areas such as cardiovascular disease, neurological disease, oncology etc. Identifying protein expression changes that associate with diseases or disease sub-types, or that are modulated in response to drug treatments can help reveal the underlying biological mechanisms and pathways involved, providing invaluable information for translational applications. The examples presented also involved a range of different sample matrices in addition to plasma, showing the versatility of the panel for a broad range of studies.

Using Olink's mouse panel could help to characterize mouse models more thoroughly and better understand pathophysiological processes and drug responses, improving the chances of successful transition from an animal model system to human clinical application.

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