Gut mucosal gene expression in inflammatory bowel disease

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chapter NINE

Future Perspectives

Developments in single-cell research

Similar to the developments achieved in GWAS and bulk RNA sequencing, the scale of single-cell RNA sequencing is constantly growing through ongoing cheaper and more efficient data generation. Future studies will allow us to conduct meta-analyses, which will highlight robust results. Standardization of tissue dissociation and library preparation methods is a prerequisite to perform these analyses, as batch effects are easily induced and may mask the true findings (**chapter 6**). Furthermore, the implementation of sample cryopreservation will provide higher throughput and more comparable results while limiting possible batch effects.

Studies that use paired inflamed and non-inflamed tissues[1,2] provide crucial information on what transcriptional and cell-compositional changes occur upon inflammation. Longitudinal data will add to the knowledge by answering, for example, the questions whether each individual disease exacerbation within one IBD patient is mediated by the same cell types and inflammatory cytokines or not, and whether inflamed and non-inflamed tissues undergo changes after exacerbations and received treatment, for example with anti-TNFα antibodies. Clarifying these processes will provide knowledge on which therapies to choose when for which patient. This will likely improve the current 'standard' biologic drug efficacy of 30%-40% for inducing and maintaing remission.

Following the developments of bulk mRNAseq in the field of IBD, the future of single-cell sequencing lies in the integration of different data layers, so-called 'multi-omics' (**Figure 1**). The first step in this process will be a bioinformatic exploration of omics data integration. Confirmation of the findings should be done in the wet lab, using genetically modified cells in a multi-layer model system to test their functions. Placing single-cell transcriptome and protein expression data layers into in-situ biological context has become possible recently through spatial transcriptomics. With this novel technique, physical and chemical interactions between cells and signaling molecules can be determined by combining the position of mRNA molecules on a tissue slide image with the corresponding sequencing data. The downsides of this technique are that it cannot (yet) be applied at high-throughput, and that the spatial resolution is not as precise that it covers single cells yet. However, rapid developments are to be expected as large companies such as 10x Genomics have commercialized the method. Finally, using current and future generated single-cell sequencing data, we may be able to develop thorough cell deconvolution methods for RNAseq[3], enabling single cell level analysis of the many large RNAseq datasets that are currently available.

With these methods, the full wealth of RNA sequencing data that has been generated over the years can be used for cell type specific analysis, leaving scRNAseq as a method

for confirming findings from bulk data. In the coming years, our group will question single cell eQTLs to confirm the findings retrieved from bulk data in **chapter 4**. This is promising to give an insight into genetically pre-defined differences in, for example, immune response in IBD.

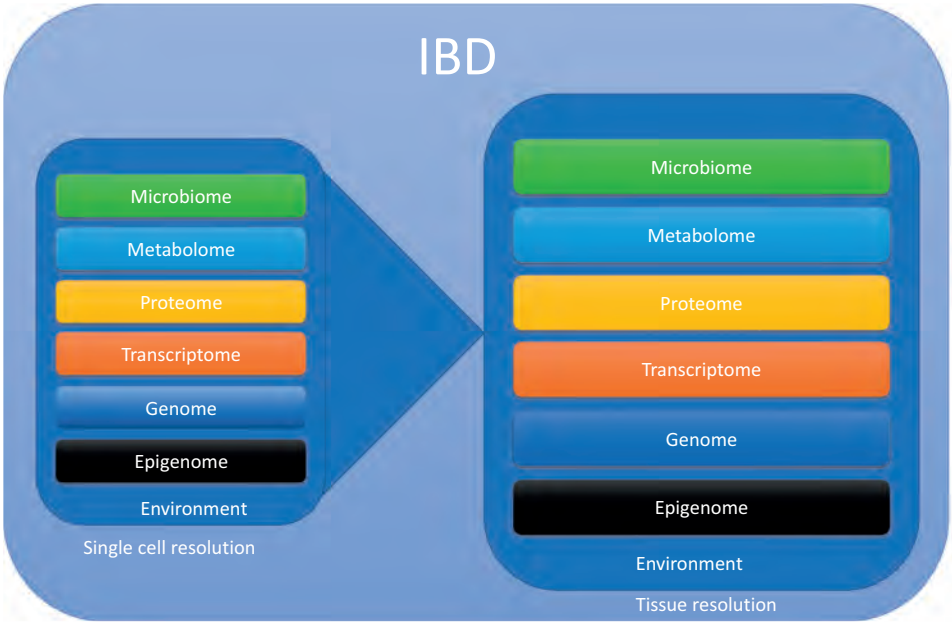

Figure 1. Integration of single cell multi-omics and tissue level multi-omics will eventually explain the pathology of IBD

The proof of concept: Physical modeling

Most results presented in this thesis are descriptive due to the explorative nature of cross-sectional mRNAsequencing data analyses, although ideally, one would functionally confirm the findings in the wet lab. Promising physical modeling systems have been developed recently to do so. Among them are small human-originating systems called 'organoids' (mini organs[4]), which represent a 3D model of an organ generated from human primary stem cells. Organoids can be produced on a large scale and can be created with a specific patient-derived genetic background using for example patient-derived cells. As a result, they have proven to be a useful drug screening platform in cancer research[5]. However, organoids exist of epithelial cell types only and, therefore, do not allow studying the epithelial-immune cell interaction within their 3D structures. This significantly limits the use of organoids for drug screening for IBD. More recently, the (multi-)organ test system 'organ on a chip'[6] has been introduced. The intestinal variant

'gut on a chip', which is under development in our Groningen lab, is a promising model to study complex interactions. The model combines immune cells, (organoid-derived) epithelium, microbiota and a continuous fluid flow, enabling exchange between the 'blood', the 'gut' and the 'luminal' compartments. Being a fairly new technology, the high costs of the current chip models and the labor intensiveness prevent its use in a high-throughput setting. Once these constraints are lifted, the 'organ on a chip' is expected to yield a thorough confirmation of the big data research findings.

From bench to bed: personalized treatment for a heterogeneous disease

The interactions between genetic variants and environmental factors, as well as the differences in response to medication between IBD patients, directly underline the complexity and variability of the disease. This thesis amongst others[7–9] discusses that IBD does not 'simply' cover the two diseases UC and CD, but instead is a continuum of inflammatory diseases, for which personalized treatment is required.

To some extent personalized treatment, although it may sound futuristic, is already implemented in today's IBD clinic. The simplest and most common form of personalized treatment is monitoring trough levels of infliximab, for example, in patients and then adjusting the drug to a patient-specific therapeutic dose. A more advanced method is making use of the fact that the side effects, toxicity and effectiveness of treatments are (in part) dependent on the genetic background. Thiopurine-induced myelosuppression, for example, was found to be more prevalent in people carrying genetic variations in the *TPMT* gene region. Now, medical centers worldwide test for these genetic variants in patients experiencing myelosuppression upon receiving the thiopurine therapy. For a handful of other IBD drugs, genetic association studies have identified variants predisposing for adverse drug reactions. However, this knowledge is so far only limitedly used in clinical practice. Recently, our group has demonstrated the clinical use of a genetic passport, in which all current knowledge on the interactions between genetics, drug toxicity and effectivity can be incorporated, and showed its cost-effectiveness[10–12]. It is not far in the future that medical centers worldwide will scan a patient's genome for genetic variants at presentation in order to enlarge drug effectivity and to avoid predicted side effects based on predicted values. With more data becoming available every day, this passport can be regularly updated to provide maximum benefits.

Defining the working mechanisms of therapeutics to explain differences in efficacy

In IBD treatment, therapeutic failure is a recurring issue, causing a burden for the patient and excessive costs for the health care system[13]. Therapy prescription that is based on a predicted response (so-called personalized or precision medicine) is a key to lower this burden. Association analyses between response status and patient-derived cellular and molecular data may provide molecular and phenotypic signatures to predict responders based on previously known data. At the same time, knowledge on the exact mechanisms of action of therapeutics may benefit their use. Altogether, this may shine a light on why some patients lose response after years of effective therapy or do not respond at all.

Drug working mechanisms

Biologics are marketed with a clear theoretic working mechanism. These drugs are generally designed to modify specific disease pathways, making use of the latest functional mechanistic insights[14]. Genetic variants, and especially those that are in the coding part of the genome, are an attractive target for the pharmaceutical industry to develop new biologicals for. As an example: after the *JAK2* gene and the *ITGA4* gene have been identified to harbor risk alleles for IBD, two drugs (JAK2-inhibitor filgotinib and α4β7-blocker vedolizumab) have entered clinical testing and are prescribed to treat IBD. Vedolizumab is assumed to have an elegant, yet simple mechanism of action: by blocking the integrin α4β7 expressed on the cell surface of T cells, it prevents T cell entry into the gut mucosa[15]. In practice, however, the drug binds to a wider range of cells and its working mechanism may be more complex than initially thought[16] (**Figure 2**). This complexity has not been well explored yet, and may explain the encountered side effects and inter-individual differences between responders and non-responders.

Response prediction

Genetic variation is not the sole factor that determines drug response. Recently, it has been found that patients who failed to respond to one anti-TNF therapy, have a lower chance to respond to a second biological therapy[17]. Using scRNAseq, a cellular enrichment profile pre-treatment (a so-called "GIMATS module" that involves inflammatory macrophages, activated DCs, highly activated T cells, IgG plasma cells, activated fibroblasts, and *ACKR1*-activated endothelial cells) was found to mark a higher chance of anti-TNF therapy failure in CD patients. These patients might have benefitted from receiving a therapy other than anti-TNF as their first therapy. Although not directly applicable in clinical practice, findings such as the GIMATS module may help to understand the working mechanisms that underlie the response to anti-TNF treatment[18].

In a preliminary exploration we found that the abundance of $CD3^+$ T cells in the blood pre-treatment varies depending on response to the biological vedolizumab (**Figure 2**).

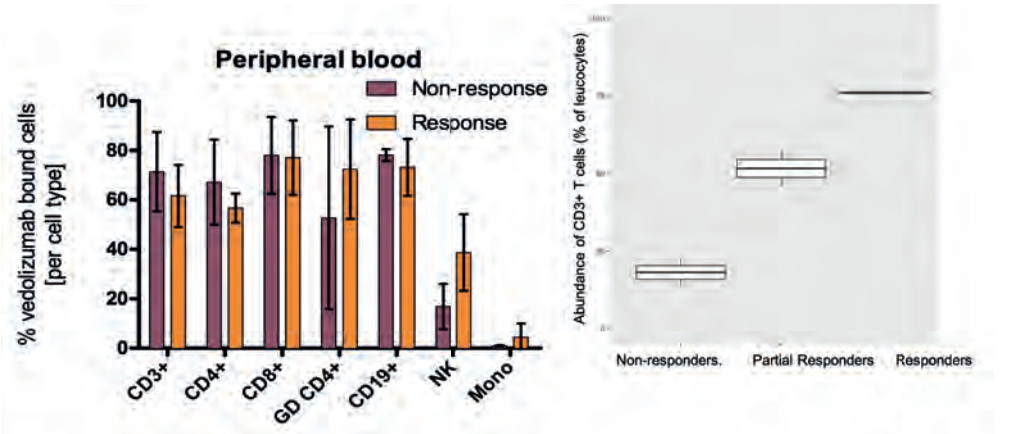

Figure 2. The drug vedolizumab was tagged with a fluorescent antibody and IBD patients' blood cells were stained with this and other cell markers. Expression of vedolizumab of multiple cell types was assessed through flow cytometry, and expression of vedolizumab was seen on T cells, but also on B cells, NK cells and monocytes. Patients were given vedolizumab and response was characterized according to a combination of doctor's opinion, continuation with the drug, endoscopy results and inflammatory parameters. Responders showed a trend towards higher abundance of T cells pre-treatment. *Courtesy of Amber Bangma*

In addition, drug components may be metabolized by human microbiota in a personalized way[19]. Following up on these findings, in ongoing research we aim to describe the full drug-response homeostasis to vedolizumab using single-cell sequencing of blood and gut cells and the corresponding microbiomes of patients receiving the drug. This will pave the road for response prediction and development of patient-tailored therapy plans.

Towards better therapies in the future

Improvements in the use of current treatments for IBD can be achieved by patient stratification based on the likelihood of therapy response. Studies that search for factors that influence treatment response are scarce, and the results are often not fit for direct, daily clinical application. Considering that multiple disease pathways play a role in IBD, it has been proposed to combine multiple (biologic) drugs targeting diverse disease pathways. One could think of a combination of inhibiting tissue entry of leukocytes by vedolizumab and inhibiting sustained inflammation by anti-TNF. This is a method that is not (yet) part of daily practice. Studies investigating the use of multiple therapies to tackle more than one disease pathway, however, have started to emerge, and their

outcomes are awaited[14]. Expanding our view to the field of oncology, where personal treatment plans combining multiple medications have been the standard of care for years, may help to implement a similar treatment approach in IBD. Oncology patients are stratified based on hormone sensibility and other molecular aspects of the tumor. Digital therapy assistants, needing no more than a few patient and tumor characteristics, are able to predict therapy effectiveness and suggest a therapeutic plan. The upcoming study results will show whether the advantages of combining treatments (*i.e.* potential higher effectivity) outweigh the disadvantages (*i.e.* higher costs and possible side effects) in IBD.

Next to pharmacological therapy, cell-based therapy demands attention of both clinics and research. In oncology, Chimeric Antigen Receptor (CAR)-T cells, for example, have shown to be effective as an anti-tumor therapy. Inspired by this, CAR-Tregs have been developed and reported to be effective in murine colitis, making them a potential therapeutic for human colitis[20]. These regulatory T cells can be programmed to target specific cells based on the expressed cell surface receptors. In addition, stem cell-based therapies have been implemented for some forms of IBD, generating promising clinical results[21].

In conclusion, there is a world of developments ongoing that will undoubtedly improve IBD care. I envision that future choice of therapy for IBD will be tailored to the patient based on molecular and physical response predictors. With the advances in bioinformatics, all (known) factors involved in disease pathology will be incorporated in disease models, thereby creating networks which visualize pathology processes, indicate target cells, genes and dietary components to treat (and hopefully cure) IBD. The most short-term clinical improvements can be made by providing therapies for targeted patient subgroups with high predicted response.

It is unacceptable that the current 'standard' of clinical effect for therapeutics in IBD is only 30%-40%: we must aim higher.

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