Skin and Bones

CHAPTER 7
Valorisation

The relevance of a diagnosis

In this thesis, we identified a novel homozygous mutation in *MMP14* in two patients with a multisystem disorder (see **Chapter 1**) and subsequently confirmed its pathogenicity at the cellular level (see **Chapter 2**). This enabled us to provide the patients with a final diagnosis: Winchester syndrome (WS). Although this diagnosis does not alter the therapy that the patients are currently receiving, it is nevertheless important for several reasons. Firstly, it becomes possible to provide the patients with adequate information about their disorder and the underlying cause. This not only helps the patients in understanding of, but may also improve coping with their symptoms [1, 2]. Secondly, a diagnosis can focus additional examinations based on tissues and organ systems known to be involved in the particular disorder. Third, as WS is an autosomal recessive disorder, confirming the molecular diagnosis provides the opportunity to offer more precise genetic counselling to the patients (and partners), informing them about inheritance and the potential risk to their future offspring [1].

The value of investigating rare genetic disorders

WS is an ultra-rare autosomal recessive disorder; the molecular diagnosis has only been confirmed in four patients worldwide [3-5, see **Chapter 1**]. The clinically overlapping and genetically related skeletal dysplasias Frank-Ter Haar syndrome (FTHS) and multicentric osteolysis, nodulosis, and arthropathy (MONA) only add a limited number of patients [6-9]. Spending significant amounts of time, effort and taxpayer's money on investigating these rare disorders may, therefore, seem only to benefit a handful of affected patients. However, the power of studying rare monogenetic disorders lays not so much in the number of patients with that exact diagnosis, but rather in the unique opportunity the direct genotype-phenotype correlation offers to study more common pathological processes from a unique and yet unexplored angle. This approach has previously been demonstrated successful and has contributed to major advanced in various fields. For instance, many genes now known to regulate osteoblast and osteoclast differentiation and function, including Runx2, Msx2, Sox9, FGFR1-3, and cathepsin K, were first identified by studying monogenetic disorders [10]. Moreover, studying rare monogenetic disorders can lead to the identification of novel therapeutic targets. By this, studying rare genetic disorders can be beneficial to patients suffering from more prevalent disorders [5].

Winchester syndrome as a model for acne and reduced bone density

WS is a skeletal dysplasia characterised by a progressive generalised reduction of bone density, thoracic kyphosis, craniofacial dysmorphology and severe nodulocystic acne with dermal fibrosis and excessive scar formation [3, 4]. We are therefore convinced that WS can accurately model reduced bone density and acne (scarring). As mentioned

above, there is significant clinical overlap between WS, FTHS, and MONA [6-9]. The protein products of the involved genes *MMP14*, *SH3PXD2B,* and *MMP2,* respectively, cooperate in extracellular matrix (ECM) remodelling and invasive cell motility (see **Chapter 1**) [9, 11-15]. As such, this led to innovative lines of research studying the pathogenesis of acne and reduced bone density from a novel angle.

Acne affects a significant part of the population and novel therapeutic options are needed

Acne vulgaris is the most common skin disorder in the world and one of the most common ailments overall. In developing countries, it affects almost 100% of adolescents [16]. Although usually mild and self-limiting, it is a chronic disease that is typically present for many years. In addition, acne is moderate-to-severe in 15-20% of patients and often persists into adulthood, with an estimated prevalence of 12% in adult females [17]. Apart from physical symptoms that are present while acne is fulminant, including soreness, itching, and pain, moderate-to-severe acne can leave disfiguring scars [16, 18]. Furthermore, acne can have a large impact on the patient's quality of life and is often associated with significant psychosocial morbidity such as self-consciousness and anxiety in social interaction, even when the acne itself is comparatively mild [18]. This psychological burden can be explained by the involvement of directly visible skin and occurance during puberty, an age that is crucial for building confidence and self-esteem, while popular culture furthermore emphasises the importance of flawless skin [18]. The psychosocial comorbidity moreover has been demonstrated to have a negative impact on school and work performance. In the US alone, it was estimated that annually over 3 billion dollars are lost to the costs of treatment and reduced productivity [16].
Although mild acne can be effectively addressed with topical drugs, moderate-to-severe disease requires systemic therapy to prevent excessive scar formation [18]. However, current treatment options are limited and their use restricted by their side effects [19, 20]. No novel drug classes are being developed for moderate-to-severe acne since there is a profound lack of insight into acne pathogenesis [21, 22]. As such, there is a significant unmet medical need.

In vitro 3D-culture for development of novel anti-acne therapeutics

As stressed above, novel anti-acne therapeutics need to be developed. Acne is characterised by the presence of comedones, which are cystic sebaceous glands, accompanied by inflammation around these structures [18]. The sebaceous gland is in essence a hollow, branched mini-organ. For normal morphogenesis of branching structures, ECM remodelling is essential [23]. Our WS patients demonstrated defective ECM remodelling and moreover, have a loss-of-function mutation in *MMP14* (see **Chapter**

2), a central player in pericellular ECM degradation [24]. This urged us to explore the possible involvement of disturbed lumen formation, as a consequence of impaired ECM remodelling, in the pathogenesis of acne. In a pilot study, we used a simple, well-established *in vitro* 3D cell culture model to confirm a crucial role of MMP14 and SH3PXD2B in lumen formation by epithelial cells [25]. Moreover, we demonstrated that therapeutic plasma concentrations of 13-cis RA have a stimulatory effect on lumen formation (see **Chapter 3**). The latter further highlighted the suitability of this *in vitro* model for assessing the effects of drug treatment. These promising results led to two subsequent studies. Firstly, as part of a multi-million dollar industry collaboration with a major international cosmetics producer, the *in vitro* model used in our pilot study was further developed for use with sebocytes. As this was successful, this novel sebocyte 3D model was subsequently used to assess the effects of existing (e.g. 13-cis RA) and novel drugs on organoid formation and sebum production. Secondly, collaboration was started with the University of Dundee Drug Discovery Unit. This latter project highlighted the potential of this *in vitro* model: upscaling with semi-automated analysis for drug screening, as reported before [25].

The zebrafish is a valuable model organism for skin research

The aforementioned *in vitro* 3D cell culture model offers the possibility to study lumen formation and the effect of prospective therapeutic compounds thereon. However, other aspects of acne, such as scarring, are hard – if not impossible – to model *in vitro*. To study these aspects, ultimately an organismal model is needed. Although *MMP14* mutant and knockout (KO) mice have been previously generated, mice are unsuitable for drug screening for a variety of reasons, such as high cost and the consequent inability to analyse large numbers of animals. A more suitable model organism that does not have these limitations is the zebrafish [26, 27]. Besides its use in genetic studies, the zebrafish is a powerful model organism for skin research. Adult zebrafish have dermal scales that are covered by a non-keratinizing epidermis. In addition, zebrafish do not have sebaceous glands, but instead harbour mucous glands [28, 29]. Apart from these adaptations to aquatic life, the architecture of zebrafish skin is remarkably similar to that of mammals. Similar to humans, zebrafish epidermis consists of multiple cell layers that moreover correspond to their human counterparts including the stratum basale, spinosum and granulosum [28, 29]. The epidermis is separated from the underlying dermis, containing the scales, by a basement membrane zone. Another attractive feature of zebrafish is their rapid external embryonic development; zebrafish form a fully functional multi-layered skin in only six days [29]. During this process, zebrafish are optically transparent and hence suitable for live *in vivo* microscopy experiments. Moreover, wound healing in zebrafish skin follows a similar basic sequence of events compared to mammalian skin [29, 30]. The causative gene of WS is conserved between human and zebrafish and in this thesis, we presented the first *mmp14a/b* KO zebrafish

model (see **Chapter 4**). We furthermore demonstrated that the phenotype of our fish recapitulates key aspects of the human pathology, suggestive of defective collagen remodelling as the underlying central problem. Therefore, we believe our fish can serve as a useful *in vivo* model for cutaneous tissue remodelling and scarring and designed a novel project to be carried out by the A*STAR Skin Research Institute of Singapore in collaboration with the NTU Lee Kong Chian School of Medicine. Within this project, which will commence in September 2018, collagen remodelling will be assessed during cutaneous wound repair in our mutant fish. Subsequently, this will be used as readout for the effect of different commercially available and newly generated therapeutics. As such, this model may lead to the development of novel strategies targeting acne scarring.

Reduced bone density is an increasing problem

The skeletal dysplasias, including the multicentric osteolyses WS, FTHS, and MONA, are a heterogeneous group of over 450 inheritable disorders characterised by a generalised abnormal growth and development of bone and cartilage [31, 32]. Although the individual dysplasias are rare, a recent study conducted in South America analysing over 1.5 million births demonstrated that collectively the prevalence of skeletal dysplasia is 3.2 per 10,000 births [32]. A large number of skeletal dysplasias, including WS, present with reduced bone density [33]. In the general population, osteoporosis (defined by bone mineral density more than 2.5 standard deviations below the average of a young adult) is an increasing worldwide problem [34]. In the Netherlands, the prevalence of osteoporosis has been estimated to encompass 431,400 people in 2015, the majority of which are postmenopausal women [35]. In this subgroup, about 40% is affected by osteoporosis [36]. A reduction in bone density increases the risk of fractures: patients with osteoporosis have a lifetime risk for fractures of 40%, primarily of the spine, hip and wrist [34, 36]. These fractures often result in chronic pain and/or loss of mobility and autonomy, and can have a drastic effect on the patient's quality of life [34, 36]. In addition, fractures of the hip and spine are associated with a significant mortality as a result of complications due to hospitalisation [36]. In 2010, the annual health care costs of osteoporosis-related fractures in the Netherlands amounted to nearly 200 million euros [34]. As the prevalence of osteoporosis increases with age and the Dutch population is ageing, the number of osteoporosis-related fractures and accompanying costs are expected to increase in the future [35, 36]. Although present therapies are efficient, most have side effects limiting their long-term adherence [36]. Therefore, development of novel therapeutics is needed.

Zebrafish are invaluable in bone research

For the development of drugs targeting bone density, an organismal model is needed. The zebrafish is a well-established model organism for vertebrate development. Zebrafish form all major types of skeletal cells and tissues found in higher vertebrates including humans [37-39]. Furthermore, bone formation and remodelling is similar in zebrafish and humans, and the pathways regulating these processes are well conserved [37, 39-41]. As such, the zebrafish is a good model organism for human bone disorders. Our zebrafish model of WS had an overt skeletal phenotype that included decreased bone density. Therefore, we believe our WS zebrafish model can be used for testing novel therapeutic strategies aimed at improving skeletal development and increasing bone density. As part of the planned project mentioned above, our mutant fish will serve as a model to test the effect on bone development and bone density of existing therapeutics currently not used for this purpose. This collaboration will enable the development of novel therapeutic strategies addressing two common conditions. Due to its focus on tissue remodelling and invasion, there is considerable potential for spin-off into other common pathological conditions such as scarring and cancer. In addition, the workflow developed in the program can be applied to other (skin) disorders.

Conclusion

In conclusion, studying the molecular basis of rare disorders such as Winchester syndrome is important for two main reasons. Firstly, providing the affected patients with a definite diagnosis is important to improve coping with their symptoms, focussing clinical follow-up examinations and offering genetic counselling. Secondly, rare disorders can provide novel insights into more common pathologies. As such, the models used and developed in this thesis have been or will be subsequently used in academic and industry collaborations to develop novel therapeutic strategies for two common disorders: acne vulgaris and reduced bone density.

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