Hidradenitis suppurativa (HS)

Illustration by Simon Abranowicz [1].

Subject Module Project in Medical Biology

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Abstract

Hidradenitis suppurativa (HS) is an autoinflammatory skin disease affecting roughly 1-4% worldwide. Symptoms of HS include abscesses, sinus tracts and scarring, which can cause immense pain and discomfort for patients. The disease impacts the pilosebaceous units in apocrine gland-bearing regions with skin-to-skin contact. Thus far, the cause of HS is unknown, however, hyperkeratosis of the follicular epithelium is most likely the first event in the development of HS lesions. Current treatment options range from topical- and systemic treatments, light therapy, and biological agents to surgical intervention. These therapies do not warrant optimal results; therefore, the aim of this report is to choose new potential drug targets. In that respect, we present specific isoforms of aquaporins (AQP3 and AQP5) and matrix metalloproteinases (MMP-2, MMP-8, and MMP-9) as possible targets that may be involved in the early and late stages of HS, respectively. Although the particular roles of AQP3 and AQP5 have not yet been determined, these aquaporins are involved in the proliferation and differentiation of keratinocytes. Furthermore, studies have discovered that AQP5 is upregulated in HS skin. To determine the function and gene expression of AQP3 and AQP5 in HS and healthy skin, we suggest using the modified MTT assay and one-step RT-(q)PCR. The other targets, MMP-2, MMP-8, and MMP-9 are involved in the degradation of the extracellular matrix. Studies have measured elevated levels of these MMPs in HS lesional skin, thereby prompting speculation to their involvement in the development of nodules, sinus tracts and abscesses. To determine the presence of MMP-2, MMP-8, and MMP-9, we suggest using gel zymography and ELISA to analyze *ex vivo* skin from HS patients. By using these methods, the amount of the target MMPs in the samples as well as the binding affinity of an antibody MMP inhibitor can be determined. In conclusion, this report underlines the need for continuing the research of HS to obtain optimal treatments for patients suffering from the disease.

Resume

Hidradenitis suppurativa (HS) er en auto-inflammatorisk hudsygdom der rammer omkring 1-4% på verdensplan. Symptomer på HS omfatter bylder, *sinus tracts* og ardannelse, der kan forårsage stor smerte og ubehag for patienterne. Sygdommen påvirker *pilosebaceous* enheder i apokrine kirtelbærende områder med hud-mod-hud kontakt. Årsagen til HS er indtil videre ukendt, men hyperkeratose af follikulær epitel er højst sandsynligt den første begivenhed i udviklingen af HS-læsioner. De nuværende behandlingsmuligheder dækker over topisk- og systematisk behandling, lysterapi, biologisk behandling med ADA, samt kirurgiske indgreb. Disse behandlinger garanterer ikke optimale resultater, derfor er formålet med dette projekt at udvælge nye potentielle lægemiddel-*targets*. I den henseende præsenterer vi specifikke isoformer af aquaporiner (AQP3 og AQP5) og matrix metalloproteinaser (MMP-2, MMP-8 og MMP-9) som mulige *targets*, der kan være involveret i henholdsvis de tidlige- og sene stadier af HS. Selvom de specifikke roller af AQP3 og AQP5 endnu ikke er bestemt, er disse aquaporiner involveret i proliferation og differentiering af keratinocytter. Desuden har undersøgelser vist, at AQP5 er opreguleret i HS hud. For at bestemme funktionen samt genekspressionen af AQP3 og AQP5 i HS- og sund hud, foreslår vi at bruge *modified MTT-assay* samt *one-step RT-(q)PCR*. De andre *targets*, MMP-2, MMP-8 og MMP-9, er involveret i nedbrydningen af den ekstracellulære matrix. Undersøgelser har påvist forhøjede niveauer af disse MMP'er i HS-læsional hud, hvilket giver anledning til spekulationer om deres involvering i udviklingen af knuder, *sinus tracts* og bylder. For at bestemme tilstedeværelsen af MMP-2, MMP-8 og MMP-9, foreslår vi at bruge *gel-zymography* og *ELISA* til at analysere *ex vivo* hud fra HS-patienter. Ved at bruge disse metoder kan mængden af MMP i prøverne, såvel som bindingsaffiniteten af en antistof-MMP-hæmmer, bestemmes. Afslutningsvis understreger denne rapport behovet for yderligere forskning af HS for at opnå optimale behandlinger til patienter, der lider af sygdommen.

Preface

This report is a fourth semester project completed by six students from Roskilde University: Anna Serop Larsen, Anne-Sofie Boelt Bruhn, Johanne Holmgaard, Malou Søndberg Svendsen, Mathilde Ellitsgaard and Sarah Toftebo Birkedal.

This report examines the pathogenesis of hidradenitis suppurativa and the associated problems regarding the lack of efficient treatment. Based on the current knowledge of the underlying mechanisms of HS, we discuss two potential drug targets, aquaporins and matrix metalloproteinases.

The report was completed in May 2022.

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Introduction

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic debilitating inflammatory skin disease of the hair follicle. HS is characterized by recurrent deep-seated and painful inflamed lesions, nodules/abscesses, sinus tracts and draining fistulas in the apocrine gland-bearing areas of the body [2]–[7]. These outbreaks mostly occur on body sites with skin-to-skin contact, such as the axilla, groin, anogenital region and inframammary folds [2]–[4], [8]. Global prevalence estimates of HS range from 1-4% depending on several factors, such as ethnic background and the method of data collection [3], [5], [9], [10]. In general, women are more often affected by the disease compared to men in a ratio of 3:1 [11]. In addition, HS predominantly affects women in Western countries [5], [12]. The symptoms of HS usually occur after puberty, improve during pregnancy and get worse around menses [11]. Studies show that approximately 34-42% of patients have a family member with HS, indicating that genetics may impact the development of the disease [11], [13], [14]. Besides the physical complications, HS can also affect the patient's mental health [2]. The psychological impacts such as poor body perception and self-esteem increase the risk of developing anxiety and depression, which can further worsen the quality of life [2], [15], [16]. This underlines the importance of studying the disease to a greater extent, focusing on better treatment options for patients with HS. Thus far, there is no cure for HS, which is partly due to the fact that the pathogenesis of HS is not fully understood and that several factors may affect the disease [17]. Treatments such as oral antibiotics and surgery can manage the symptoms, however, the response rates vary. Therefore, new and effective treatments are needed. Based on the knowledge behind the underlying mechanisms of HS, this report aims to suggest new drug targets: aquaporins (AQP3 and AQP5) and matrix metalloproteinases (MMP-2, MMP-8, and MMP-9). In addition, we will propose experiments that may determine the importance of these specific targets in the development of HS.

Problem Definition

How is the current understanding of hidradenitis suppurativa (HS) problematic? Which targets could be relevant for the treatment of HS and how can this relevance be determined?

Background

The Immune System

The immune system can be divided into the innate and the adaptive immune system. The innate immune system acts as a first-line defense against microorganisms, whereas the adaptive immune system provides better protection against reinfections [18]. Basic functions of the immune system are the recognition of foreign antigens and the elimination of these, the development of tolerance to self-antigens, and immunologic memory [19]. Immunological memory is one of the most central properties of the immune system, as it confers spread limitations and protects against reinfections. Antibodies traveling in the blood administers the immunological memory throughout the body [18].

Barrier Defenses of The Innate Immune System

The innate immune system includes barriers that keep harmful pathogens from entering the body. The barriers can be divided into physical barriers and defense mechanisms. The physical barriers comprise the skin, the gastrointestinal and respiratory tracts while the defense mechanisms include mucous, gastric acid and sweat. The skin is able to kill pathogens due to its acidic and dry surface. The regions on the body that are not protected by skin, like the eyes and mucous membranes, will be protected by tears and mucous secretions that trap pathogens. Despite these barriers, pathogens may still enter the body, further triggering the components of the innate immune system [20].

Components of the Innate Immune System

The innate immune system is referred to as non-specific, as it provides protection against pathogens without needing preconditioning from the environment [20]. Thereby, the innate immune system responds similarly to all germs and foreign substances. The innate immune system is encoded into the germline and passed down from generation to generation. The major components of the innate immune system are effector molecules and cellular components. Cellular components include macrophages, dendritic cells, natural killer cells, mast cells, and granulocytes. The aforementioned cover the three cell types: neutrophils, eosinophils, and basophils. Mast cells, natural killer cells, eosinophils and basophils are not relevant for this

report, so these will not be described in further detail [20]. Nonetheless, a chart describing the components of the innate immune system and their functions can be viewed in *Appendix 1*.

Neutrophils make up half of the white blood cells in humans and phagocytose invading microbes [20]. The activation of neutrophils appears to be an important factor in the development of HS, as a high level of neutrophils is often observed in the blood of HS patients [21], [22]. Macrophages are phagocytic cells capable of ingesting and destroying microbes. These cells are long-lived and present in all kinds of tissues [23], [24]. Dendritic cells (DCs) can activate the cells of the adaptive immune system. However, they also destroy invading microbes through phagocytosis [24]. DCs and macrophages are both antigen-presenting cells (APCs). APCs display antigens on their surface and mediate crosstalk between the innate and the adaptive immune systems [20], [24]. APCs are necessary for early HS inflammation due to their expression of Toll-like receptors (TLRs), their ability to phagocytose free-keratin in the dermis, and their secretion of multiple cytokines. However, the relevance of this remains unclear [20], [22], [24], [25].

Inflammation and Innate Response

The first step for the innate immune system is to recognize a pathogen. The recognition is done by complementary pattern recognition receptors (PRRs), and these are present on the surface of APCs. PRRs, like the specific TLRs, recognize pathogen associated molecular patterns (PAMPs). PAMPs are microbial molecules such as bacterial lipopolysaccharides. The binding of TLRs with PAMPs triggers the innate immune system to release cytokines. Cytokines are chemical messengers that regulate cell differentiation, proliferation, and gene expression. There are many types of cytokines found in the innate immune system. One subclass of cytokines is interleukin (IL), which mediates leukocyte (white blood cell) interactions. ILs are involved in the crosstalk between the innate and the adaptive immune systems [24]. Nonetheless, the innate immune system may be insufficient at clearing an infection. When this happens, the adaptive immune system mobilizes a response, as illustrated in *figure 1* [20], [24].

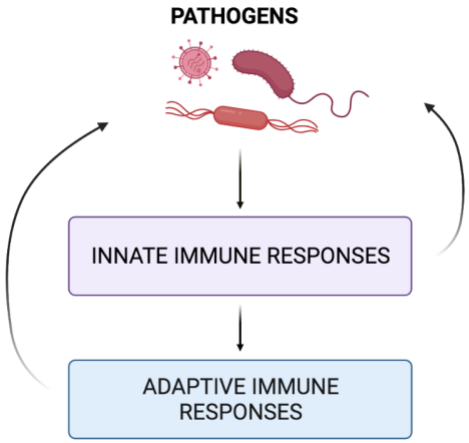

Figure 1. *Pathogens activate the innate immune system along with the adaptive immune system, helping to fight the infection. Created in BioRender.com*

The Adaptive Immune System

In contrast to the innate immune system, the adaptive immune system can discriminate between several different microorganisms and mediate responses specific to the invading pathogen [26]–[28]. These responses are damaging, which makes it crucial for the host to distinguish between foreign and self [26]. This tolerance is maintained by mechanisms that eliminate or inactivate lymphocytes expressing receptors for their own antigens [27], [28]. Lymphocytes are a type of white blood cells, consisting mainly of those derived from the thymus (T-cells) and the bone marrow (B-cells) [19]. The adaptive immune system can remember antigens that have entered the body before, so the response is usually faster and more powerful when they enter once again [27], [28]. The adaptive immune system can be divided into two divisions: humoral- and cell-mediated immunity. Humoral immunity is mediated by globulins, antibodies produced by B-cells in the blood. Antibodies have the ability to recognize certain antigens of microorganisms and bind to them, causing neutralization and elimination [27], [28]. Cell-mediated immunity is mediated by T-cells, which can be divided into cytotoxic T-cells (CTLs), T helper (Th) cells and regulatory T-cells. Regulatory T-cells suppress the growth and function of certain Th-cells and CTLs [29]. Meanwhile, CTLs kill infected cells before further replication, and Th-cells secrete cytokines after an antigen undergoes stimulation. These cytokines stimulate the proliferation and differentiation of T-cells. Furthermore, Th-cells activate other cells, such as B-cells and macrophages [27], [28]. Th-cells can differentiate into multiple subsets, including Th1, Th2, and Th17 [29].

The Integumentary System

The integumentary system is the body's outermost layer and comprises the cutaneous membrane, known as the skin, and its accessory structures [30], [31]. The skin is the largest organ in the human body, and functions as a physical barrier between the internal and external environment [31], [32]. The skin can be divided into two main components, the epidermis and dermis. In brief, the epidermis is the superficial epithelium whereas the dermis is the thick underlying layer of connective tissue, illustrated in *figure 2* [30]–[32]. The skin can also be divided into thick and thin skin depending on the thickness of the epidermis and dermis. Furthermore, there is a difference in the components of the layers. The thin skin contains sebaceous glands, hair follicles and sweat glands, see *figure 2*. In contrast, the thick skin only covers heavily worn surfaces and does not contain the aforementioned accessory structure [30], [33].

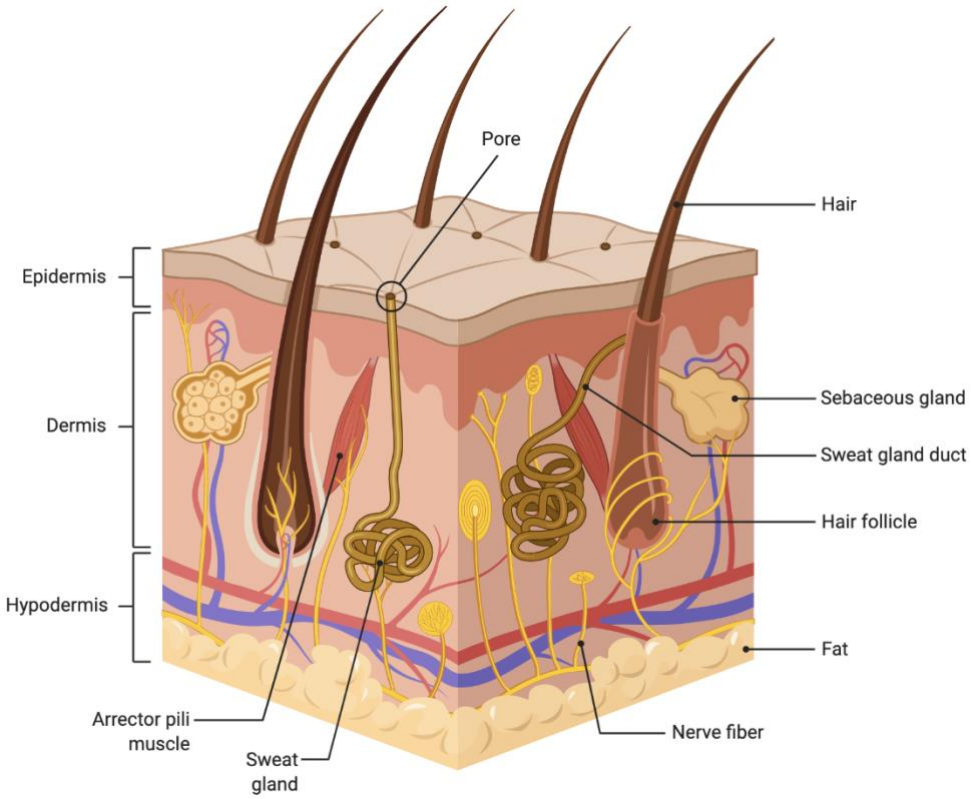

Figure 2. *The integumentary system consists of the cutaneous membrane, which includes the epidermis, dermis, and hypodermis. The accessory structures include the hair follicle, sweat glands and sebaceous gland. Fat, blood vessels and nerve fibers are located under the dermis. Created in BioRender.com*

Epidermis

The epidermis is composed of a keratinized layered squamous epithelium, containing four different cell types; langerhans cells, merkel cells, melanocytes, and keratinocytes [30]–[32]. Out of the four cell types, the keratinocytes are the primary epithelial cells, constituting around 90% of the epidermis [31]. The three other cell types are scattered among the keratinocytes in a smaller proportion [30]. Furthermore, the epidermis consists of different types of layers. In thick skin, there are five different layers whereas thin skin only contains four [30], [32]. The deepest layer is the stratum basalis (SB), which adheres to the collagen-containing basement membrane and is fixed into position through hemidesmosomes. Next comes the stratum spinosum (SS), followed by the stratum granulosum (SG) and finally the stratum corneum (SC), which is the most superficial layer [31]. The extra layer in the thick skin is the stratum lucidum. All the layers are illustrated in *figure 3* [32].

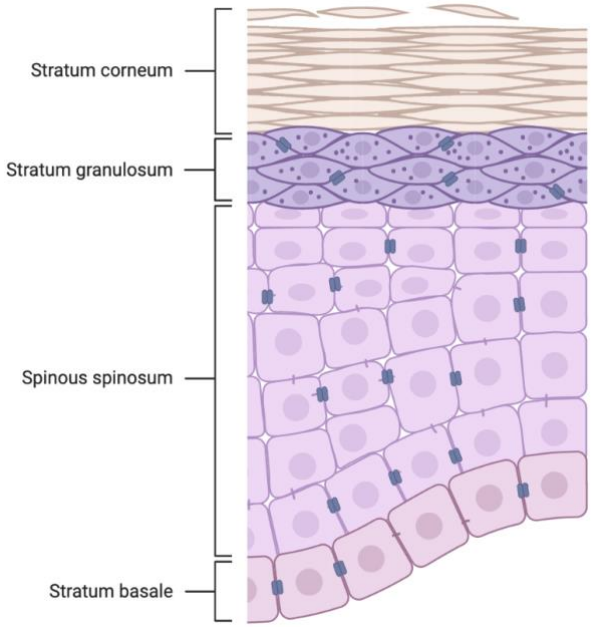

Figure 3. *Layers of epidermal cells including the stratum basale, the stratum spinosum, the stratum granulosum, and the stratum corneum. Created in BioRender.com*

The formation of the various layers of the epidermis starts in SB, through proliferation and migration of the keratinocytes. Here, the cells begin to differentiate, gradually, in the different layers, resulting in their final maturation, near the surface [30], [31]. The differentiation process is initiated in SB by the basal progenitor cells. This process is essential and very important in relation to wound healing, normal epidermal function, and circulation. In addition, dysregulation of the differentiation process may lead to the development of a number of skin

diseases such as HS [31]. As the cells differentiate and infiltrate SG, they become flatter and thinner in their structure. In addition, the cells continue to increase the production of keratin, also known as keratinization, thereby obtaining enormous amounts of keratin [30], [31], [34]. The cells in SG are important in the formation of the superficial SC and involve several proteins, such as profilaggrin, keratins, loricrin and other cornified envelope proteins [31], [34]. SC is arranged in several layers and consists of dead, densely packed flat cells with a thickened plasmalemma. The amount of layers depends on the location on the body but varies between 15 and 100 [30], [31], [34]. Under normal circumstances, SC is relatively dry. This makes growth on the surface difficult for many microorganisms and thus provides protection [30], [31].

Dermis

The dermis is located below the epidermis and is a thick connective tissue layer that helps give the skin flexibility and strength due to elastin and collagen, respectively. In addition, the dermis contains sensory receptors, accessory structures such as sweat glands, sebaceous glands, and hair strands [32]. Beneath the dermis, the subcutaneous layer is located, also known as hypodermis. It contains blood vessels and adipose tissue that supplies the dermis with nutrients and oxygen [30], [32]. Accessory structures are located in the dermis and can be divided into three main components: nails, hair, and exocrine glands. The exocrine glands can be further divided into the sebaceous glands and the sweat glands [30]. The hair protrudes through the epidermis to the surface and is mainly made up of keratinized dead cells. The hair strand resides in the hair follicle whose base lies deep in the dermal tissue. Furthermore, the follicle extends through the basement membrane, the epithelial layer and further beyond the surface of the skin, as illustrated in *figure 2* [30], [35], [36]. The hair root lies underneath SC and is anchored to the follicle itself [30], [37]. The hair follicle is anchored to the skin through the sebaceous glands and arrector pili muscle; this structural unit is also called the pilosebaceous unit [35], [36]. The sebaceous gland produces and secretes sebum, which consists of lipid droplets, into the hair follicle in order to lubricate SC via the hair shaft [38], [39]. This lubrication makes the skin and hair waterproof [39]. Interestingly, HS patients tend to have a reduced number and volume of sebaceous glands in perilesional skin [40], [41]. This could potentially result in less lubrication of SC, which will most likely lead to increased mechanical stress in body sites with skin-to-skin contact [12]. In association with the hair follicles, the apocrine glands are developed. The apocrine gland is a sweat gland that produces lipid-rich sweat which is released

into the infundibulum (the upper segment) of the associated hair follicle, near the skin surface [21], [42]. The apocrine gland differs from another type of sweat gland called the eccrine gland. This difference is due to the larger size of apocrine glands and the fact that they open into the hair follicle rather than the skin surface [21], [42]. Apocrine glands are mainly located in the chest, groin and in the anogenital and axillary regions of the body [2], [21], [42].

Etiology

The cause of HS is still unclear but likely multifactorial, meaning several predisposing factors impact the onset of the disease. These factors include genetics and lifestyle factors. The following section will review some of the factors that may contribute to the development of HS [12].

Genetics Factors

Studies by Nomura et. al. and Sabat et. al. have shown that approximately 34-42% of patients with HS report a positive family history of the disease, indicating an autosomal dominant inheritance pattern. The studies included several Chinese HS patients with positive family history. Nomura et al. and Sabat et al. found mutations in three genes; *PSENEN*, *PSEN1*, and *NCSTN*, which are located on chromosome 1. These genes encode γ-secretase and their mutant forms may cause loss of function in the components of γ-secretase, see *figure 4* [14]. Furthermore, the mutations show familiar aggregation with autosomal dominant inheritance. γ-secretase is an intramembrane protease complex with several subunits; presenilin (PSEN1), nicastrin (NCSTN), Aph-1, and Pen-2 [43]–[46]. The γ-secretase complex cleaves transmembrane proteins at residues within the transmembrane domain. The cleavage is an important step in signaling pathways, especially in the Notch pathway where the γ-secretase is related to the processing of several type I integral membrane proteins. The Notch signaling pathway is essential for normal growth, differentiation of hair follicle cells, proliferation, and cell death in the skin. Studies that include HS patients have shown that specific gene variants of the *NCSTN* reduces the amount of functional nicastrin (the protein encoded by the *NCSTN*-gene) produced in cells. This results in less protein available to act as part of the γ-secretase complex and thus impairs Notch signaling. Gratton et. al. suggest that abnormal Notch signaling may promote hyperkeratinization of the follicular epithelium, abnormal keratinocyte differentiation, disruption of the normal hair follicle cycle, and formation of follicular cysts [44], [45].

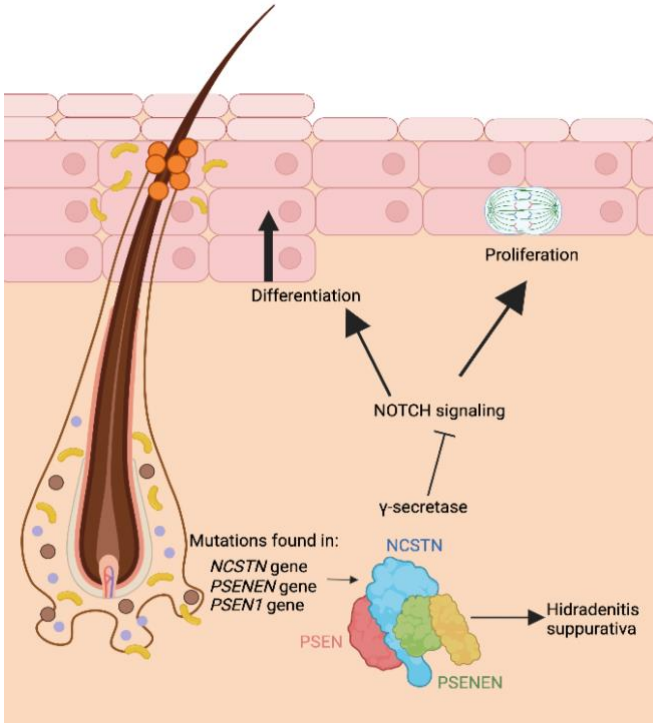

Figure 4. *Pleiotropic role of notch signaling in human skin diseases. Mutations in the genes PSENEN, PSEN1, and NCSTN cause loss of function in the components of γ-secretase and may lead to the development of HS. Inspired by figure 5 from Gratton et. al.* [47]. *Created in BioRender.com.*

Lifestyle Factors

Obesity and smoking are two major risk factors that are associated with HS. Several studies have demonstrated that a body mass index above 30 increases the risk of developing the disease [48]–[50]. Scientists have speculated that obesity has a pathogenic effect on the severity of HS. This is due to the increased skinfold friction and the low-grade systemic inflammation promoted by an imbalance in cytokines secreted by adipose tissue [12], [14]. Additionally, active smokers are more likely to be diagnosed with HS than nonsmokers [50], [51]. The nicotine from cigarettes has been related to pathogenic events in HS such as (an increase in the number of cells within the epidermis), follicular plugging, neutrophil chemotaxis, cytokine production by keratinocytes, and downregulation of antimicrobial peptides (AMPs) [49].

Pathogenesis of HS

As previously mentioned, HS affects the pilosebaceous units in apocrine gland-bearing skin. Since only body sites with skin-to-skin contact are affected, it has been suggested that repetitive mechanical stress plays an important role in the development of HS lesions [40], [52]. The first event in HS lesions is follicular occlusion due to hyperkeratosis of the infundibular epithelium, see *figure 5* [5], [40]. Hyperkeratosis is defined as the thickening of SC, which may be caused by excessive keratin in the hair follicle [53], [54]. It is possible that hyperkeratosis occurs as a result of abnormal proliferation and differentiation of keratinocytes [31], [53], [55]–[57]. As already stated, the differentiation of keratinocytes gradually increases their production of keratin. The excessive quantity of keratin and keratinocytes in the hair follicle may block the pore and thereby cause the accumulation of sebum and apocrine sweat. This leads to dilation of the hair follicle, also known as follicular plugging [5]. The resulting plug applies pressure to the cells within and surrounding the hair follicle, causing damage to these cells [5], [58]. This entails the release of cellular damage-associated molecular patterns (DAMPs), which are molecules that the surrounding cells can release in order to alert the innate immune system of the damage [5], [12], [59]. Furthermore, microbes start to penetrate the hair follicle. Ultimately, the hair follicle ruptures, releasing sebum, apocrine sweat, keratin and bacteria into the dermis [40], [58]. This seems to trigger a local inflammatory response mediated by the innate immune system [5], [46]. Macrophages from the innate immune system are activated when TLRs recognize DAMPs. As a result of the tissue being infected with bacteria, TLRs also recognize PAMPs [5]. The recognition of DAMPs and PAMPs causes an increased amount of pro-inflammatory cytokines such as IL-1β and TNF-α [12], [46].

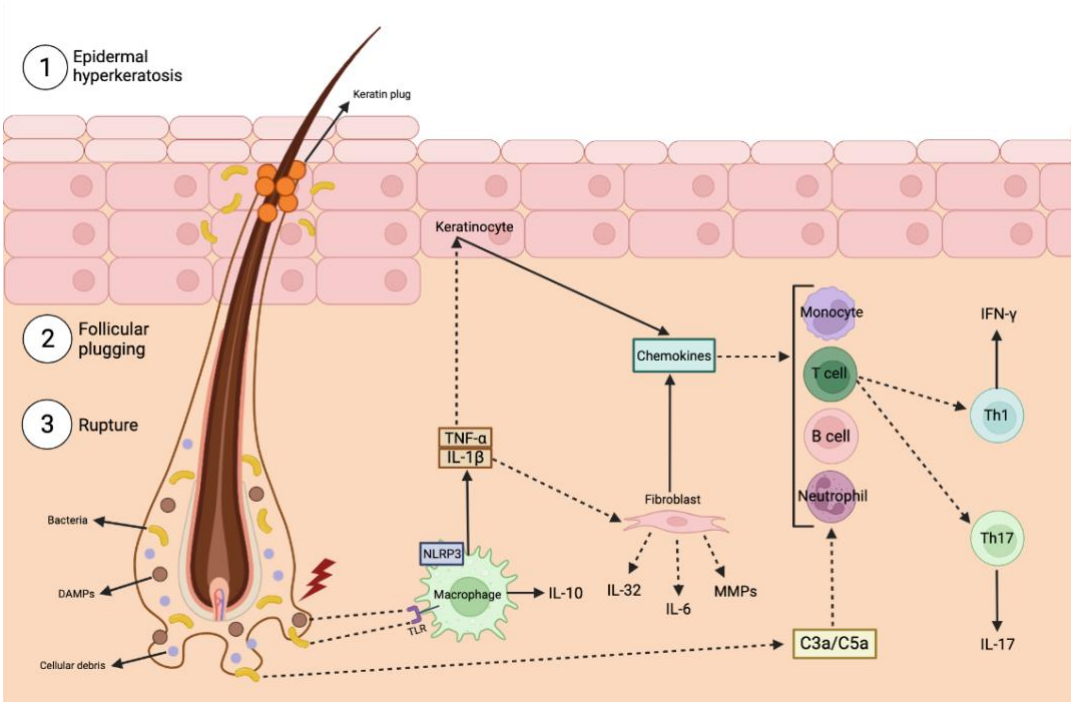

Figure 5. *Before a lesion is developed, SC undergoes hyperkeratosis possibly due to an abundance of keratin in the hair follicle. This leads to dilation of the hair follicle, also known as follicular plugging. As a result, the cells around the follicle become damaged, resulting in the release of DAMPs. Furthermore, bacteria may enter the enlarged follicle. Eventually, the hair follicle ruptures, which releases follicular content, including PAMPs. Receptors called TLRs recognize DAMPs and PAMPs, prompting macrophages to produce IL-1β and TNF-α. IL-1β and TNF-α induce the production of certain chemokines in fibroblasts and keratinocytes, respectively. These chemokines attract monocytes, T-cells, B-cells, and granulocytes (such as neutrophils). T-cells may differentiate into Th1 or Th17 cells that secrete IFN-γ and IL-17, respectively. The activation of fibroblasts also leads to secretion of IL-32, IL-6 and MMPs. Lastly, C3a and C5a are activated, resulting in the activation and recruitment of neutrophils. Inspired by figure 2 from Amat-Samaranch et al. [5]. Created in BioRender.com*

The production of IL-1β is induced through the activation of the NLRP3 inflammasome, which is a multiprotein complex of the innate immune system [5]. The NLRP3 inflammasome contains a sensor (NLRP3), an adaptor (ASC) and an effector (caspase-1). When NLRP3 senses PAMPs and DAMPs, a domain in ASC, known as the CARD domain, binds pro-caspase-1. Upon binding, pro-caspase-1 cleaves itself into its active form; caspase-1. Caspase-1 then cleaves pro-IL-1β into its active form; IL-1β [60], [61]. Finally, IL-1β induces the production of chemokines in fibroblasts, while TNF-α induces the production of chemokines in keratinocytes [12], [62]. These chemokines attract granulocytes, T-cells, B-cells, and monocytes (differentiate into macrophages and DCs). The activation of fibroblasts by IL-1β enhances secretion of IL-32, IL-6 and matrix metalloproteinases (MMPs). This results in massive tissue immune cell infiltration, formation of pus, and contributes to tissue destruction

and fibrosis [5]. MMPs are zinc dependent proteolytic metalloenzymes that are involved in the degradation of proteins in the extracellular matrix (ECM). Depending on the homology and substrate specificity, MMPs can be categorized into six main groups: gelatinases, collagenases, stromelysins, matrilysins, membrane-type MMPs and other uncategorized MMPs [63]–[66]. MMPs degrade components of the ECM due to various reasons such as paving the way for cell migration. For example, degradation of type I collagen by MMPs is essential for the migration of epithelial cells and wound healing. MMPs can also cleave the basement membrane and intercellular junctions, thereby regulating the organization of epithelial tissue [67]. Furthermore, MMPs can promote cell death, changes in proliferation, cell motility or differentiation by modifying or disabling the action of active signaling molecules. Lastly, MMPs can facilitate the recruitment and removal of inflammatory cells. By doing so, MMPs can be both anti-inflammatory and pro-inflammatory [65]. As mentioned earlier, levels of TNF-α increase when DAMPs and PAMPs are recognized by TLRs. TNF-α is produced by macrophages, DCs and T-cells [5]. Besides inducing production of specific chemokines, TNF-α also activates endothelial cells that regulate the exchange of e.g. neutrophils between the bloodstream and the surrounding tissue [12], [68]. Additionally, TNF-α increases the expression of MMPs and decreases secretion of adiponectin from adipocytes (fat cells). Adiponectin is an anti-inflammatory hormone that regulates insulin sensitivity and the metabolism of glucose. Lastly, TNF-α favors the production of Th17 cells over regulatory T-cells [5], [40]. Th17 cells produce cytokines that are necessary for the immune response to fungi and extracellular bacteria [69]. Two complement factors, C3a and C5a, have also been implicated in the development of HS. Complement factors are small proteins that play a role in both the innate and the adaptive immune system [70]. It has been established that HS patients have high blood concentrations of C5a compared to healthy individuals. C3a and C5a activate and recruit neutrophils and stimulate production of TNF-α [5], [40].

Multiple studies have measured elevated levels of interferon gamma (IFN-γ) and IL-17 mRNA in HS skin compared to healthy skin [5], [12], [71]. IFN-γ is the predominant cytokine secreted by Th1 cells and activates endothelial cells, permitting infiltration of immune cells from the bloodstream and maintaining the immune response [5]. IFN-γ stimulates the secretion of Th1-attracting chemokines, thereby creating a positive feedback loop. IFN-γ also activates tissue cells and macrophages; the latter being APCs. The activated macrophages and tissue cells strengthen the antigen presentation, which may be important for local activation of T-cells [12]. Furthermore, macrophages activated by IFN-γ synthesize IL-10, which has been observed in high levels in HS patients. IL-10 suppresses the production of pro-inflammatory cytokines,

limits T-cell activation, and thereby lowers the immune response [12]. Studies have also found correlation between dysregulation of the IL-17 pathway and the causation of chronic inflammation in autoimmune and autoinflammatory diseases, such as psoriasis and HS [12], [72]. IL-17 is a cytokine produced by Th17 cells and it mediates inflammation by inducing the production of chemokines. Additionally, IL-17 has the ability to stimulate neutrophils and macrophages, which increases the expression of IL-1β, IL-6 and TNF-α, as well as caspases and MMPs in the follicular unit and perilesional skin [5]. Another cytokine, IL-36, has a higher expression in lesional HS skin compared to the skin of healthy subjects [12], [40]. IL-36 is involved in inflammation, signaling to neutrophils and the abundance of epidermal cells [71]. Eventually, a sinus tract may be developed, see *figure 6*.

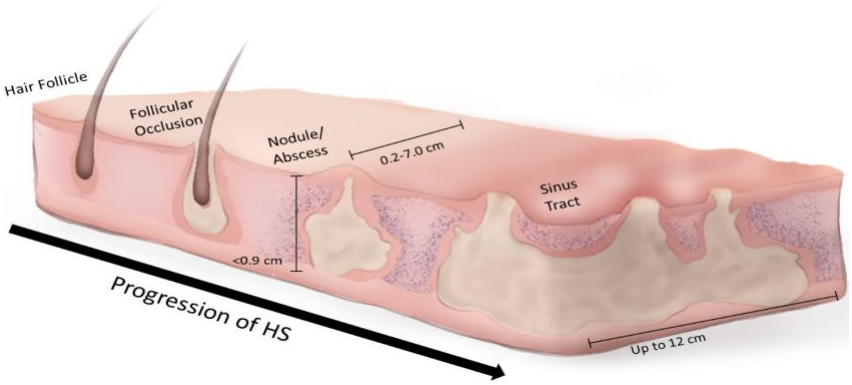

Figure 6. *Progression of HS illustrated in different stages, including follicular occlusion, formation of a nodule/abscess and a sinus tract* [73].

A possible explanation for tissue remodeling might be found in the upregulation of MMPs in HS lesions [74]. High levels of MMP-2, MMP-8, and MMP-9 have been measured in HS patients [74]–[76]. MMP-8 degrades collagen type 1, which forms collagen fibers in the dermis. The collagen fibers give the dermis endurance and flexibility [77]. Therefore, it is possible that high levels of MMP-8 cause extensive degradation of this ECM component, leading to the formation of abscesses and draining sinus tracts [75]. Measurements of MMP-2, MMP-8, and MMP-9 in the blood of HS patients show a positive correlation with HS severity as well as number of areas with inflammation and nodules. In addition, a high blood level of TNF-*α* seems to be positively correlated with high levels of MMP-2, MMP-8, and MMP-9 in HS patients. It could be assumed that bacterial products induce TNF-*α* production in lesional monocytes, macrophages, and neutrophilic granulocytes and contribute to elevated MMP-2, MMP-8 and MMP-9 expression [74]–[76].

Diagnosis and Treatment of HS

To assess the severity of HS, various scoring systems have been made [11]. The widely used Hurley staging system is a three-stage classification that depends on the severity of the disease, including Hurley I, Hurley II, and Hurley III [3]. Concisely, stage I is typified by abscess formation with no sinus tracts or scar tissue and is the most frequent stage. Stage II is characterized by recurrent abscesses with sinus tracts or scarring. Lastly, stage III is typified by larger anatomical areas, with interconnected sinus tracts and abscesses. Stage III is a severe stage, but fortunately the rarest among patients [11], [78], [79].

Medical Treatment of HS

Although there is no cure for HS, several treatment options may keep the disease under control and improve symptoms. The treatments include topical- and light therapy, systematic treatments, biological agents and surgery [52]. However, due to the lack of controlled randomized clinical trials (RCTs), it can be challenging to choose between the different treatments. In some cases, a combination of different therapies are recommended depending on the severity of the disease [5]. Patients with a mild case of HS can typically lower the symptoms by changing some lifestyle factors, which includes weight loss and smoking cessation. Mild disease can also be treated with oral antibiotics and topical treatment (medication applied to the skin) [52]. Patients with Hurley stage II-III typically fail to respond to conventional therapies, and it poses a great challenge in the treatment of this disease [5]. It is recommended that treatment-response is assessed based on improvement in clinical parameters. These include Hidradenitis Suppurativa Clinical Response (HiSCR) and Dermatology Life Quality Index (DLQI) [80]. Patients who achieve HiSCR should show a reduction of 50% in the number of inflammatory nodules and abscesses [5]. To determine which biological treatment to use, DLQI and the Hidradenitis Suppurativa Severity Score System (HS4) are used in combination. HS4 is based on both the number of affected skin areas as well as the number of lesions involved [5], [80].

Topical Treatment

Antibiotics is typically the first type of therapy used to treat HS. However, antibiotics alone will most likely not manage the condition, but may be required in combination with other treatments. It is currently difficult to compare the clinical response rates of antibiotics due to the lack of RCTs [52]. For patients with Hurley I-II stage, topical clindamycin is recommended,

supported by two RCTs [5]. Treatment with topical clindamycin has anti-inflammatory effects, as it decreases the number of inflammatory lesions and may prevent secondary infection [52]. Another topical medication is resorcinol, which is a peeling agent with anti-inflammatory effects. In a study by Amat-Samaranch et al., 85.2% of the patients treated with topical resorcinol achieved HiSCR after 12 weeks. The study included 65 HS patients with Hurley stage I-II [5].

Systemic Treatment

Systemic treatment is recommended if surgical treatment or the abovementioned topical antibiotics are ineffective. In refractory and widespread HS cases, oral antibiotics such as tetracyclines (minocycline, tetracycline, and doxycycline) are considered. In a study by Amat-Samaranch et al., 40% of the patients achieved HiSCR after 12 weeks of treatment with tetracyclines [5]. If these tetracyclines do not have the desired effect, combination therapy with oral clindamycin and rifampicin is recommended for patients with Hurley stage II-III [52], [81]. In the same study by Amat-Samaranch et al. 71% to 93% of the 187 patients achieved HiSCR after 10 weeks of treatment with clindamycin and rifampicin [5]. Another systemic treatment is intralesional corticosteroids. Amat-Samaranch et al. found that 70% of the lesions treated with intralesional corticosteroids showed complete resolution. The optimal dosage remains unclear, therefore an ongoing RCT has aimed to find the optimal dosage [5].

Targeted Biologic Therapy

As previously mentioned, TNF-α is an inflammatory cytokine playing a critical role in the pathogenesis of inflammatory diseases. From this perspective, anti-TNF-α agents have been introduced with the purpose to treat several chronic inflammatory diseases with promising results [82]. If treatment with clindamycin in combination with rifampicin does not have an effect, the anti-TNF-α antibody, adalimumab (ADA), is recommended as a therapy for patients with Hurley stage II-III [81], [83]. ADA is the only approved biological drug for the management of Hurley stage II-III. In a study by Lowe et al., less than 45% of the patients treated with ADA had a 50% reduction in HS lesions at 12 weeks [84]. If treatment with ADA is effective, it is recommended to continue this treatment until no lesions are present. If the treatment is non-effective, second- and third line treatments should be tried, which include acitretin and isotretinoin [83][84].

Laser and Surgical Treatment

Multiple laser- and light methods have been used in the treatment of HS, such as intense pulse light, long pulse Nd:YAG laser and CO_2 laser. All of these, if used alone, have been associated with recurrence [85]. In the following section, a brief explanation of the three methods will be presented followed by the surgical treatment of HS.

Intense Pulse Light Treatment

Intense pulse light (IPL) treatment uses high-energy broad-spectrum light to destroy affected hair follicles [86]. IPL devices are pretty selective, usually only damaging their target area [87]. Furthermore, this kind of light treatment is less damaging to normal tissue than continuous lasers [86]. HS patients treated with IPL for a month showed a 33% reduction in lesions [88].

CO_2 Laser

CO_2 laser is used to deroof sinuses or lesions and show effectiveness intreating scars [85]. Deroofing is a tissue-saving method where the roof or the top surface of e.g. a sinus tract or a lesion is surgically removed, mostly for treating mild to moderate HS lesions [89]. The active inflammatory nodules and sinus tracts are stained by probing with methylene blue. The dye is then spread to tracts with hidden- and minor branches too small to probe and these communicating tracts must be found for excision or deroofing [90]. A study reported that 95% of the patients whose lesions were treated with CO_2 laser, showed immediate improvement, however, 29% experienced lesion recurrence one year later [91].

Nd:YAG Laser (long pulse)

Neodymium-doped Yttrium-Aluminum-Garnet (Nd:YAG) is a laser that emits radiation in the infrared region of the electromagnetic spectrum [92]. The energy of the laser is selectively absorbed by deeper dermis and is converted into heat within the tissue [93]. Nd:YAG penetrates tissues deeper, making the depth of penetration less predictable than with the CO_2 laser [92]. Deroofing with CO_2 laser in combination with hair follicle removal by long pulse Nd:YAG laser has shown to be a minimally invasive tissue saving surgical intervention for treating HS [85]. Furthermore, the use of CO_2 laser prior to Nd:YAG enhances penetration of Nd:YAG [5]. The response rate for Nd:YAG laser has shown a 65% improvement in HS lesions after the patients received around three to four monthly treatments [94], [95].

Surgical Treatment

In mild HS, topical- or oral antibiotics alone or in combination with corticosteroids and surgery may be enough to control the disease. Yet, as the disease progresses, both surgical and systemic treatment is necessary. Recommendations for surgery on HS lesions rely on low-quality reports, where interventions such as incision and drainage of lesions only provide quick symptom relief, but with recurrence close to 100% [5]. Surgical deroofing is an adequate treatment of lesions in Hurley I-II stage, with a recurrence rate of 20-40%. Also limited local excision can be performed where separate lesions are excised - this has a similar recurrence in Hurley stage I-II patients as surgical deroofing. Surgical deroofing in combination with sinus tract excision has shown to enhance clinical results and also cause a decrease in recurrence [5]. For Hurley stage II-III patients, wide excision is primarily used. Defects following from wide excision may be sutured, closed with grafts or even left open for healing and have recurrences of 22%, 2%, and 11%, respectively [85]. The reconstruction method after wide excision greatly affects recurrence. The location of the lesion can predict outcome, with perianal, vulvar and inferior breast excisions having the higher recurrence rates [5]. In chronic severe cases, surgery is essential; it is therefore of great importance to treat the disease as early as possible, as a delay could lead to the disease developing into an uncontrolled state, making wide excision surgery a necessity [85].

Method

This report has been prepared as a literature study with National Library of Medicine used as the primary databases for our research. Throughout the literature search, we have selected studies highly relevant for the preparation of this report. Some notable keywords have recurred, including *Hidradenitis Suppurativa* and *Acne Inversa.*

The following keywords have been searched individually or in combination throughout the process working with the various sections of this report; *Treatment, Aquaporins, AQP3, AQP5, Pathogenesis, Therapy, Inflammatory Skin Disease, Matrix Metalloproteinases, Matrix Metalloproteinases Inhibitors, MMP-2, MMP-8, MMP-9, Antibody-Based Inhibitors, HDAC3 Inhibitors, Upregulation of AQP5 Expression.*

Potential Targets and Experimental Design

Target 1: Aquaporins

Considering that hyperkeratosis is one of the main events in the formation of HS lesions, the underlying mechanisms behind keratinocyte proliferation and differentiation could be of interest in future studies. Therefore, we assume that aquaporins are involved in the early stage of HS. Aquaporins are classified as transmembrane channel proteins that enable the bidirectional transport of water and small solutes, such as glycerol, across cell membranes. Mammals express 13 AQP isoforms, termed AQP0-12. Modification in AQP levels have been detected in inflammatory skin diseases such as HS. It has been suggested that the aquaporin, AQP3, plays an important role in the proliferation and early differentiation of keratinocytes, and it may therefore be a potential drug target [96], [97]. Furthermore, studies report that AQP3 is the most abundant aquaporin in the epidermis and is expressed by keratinocytes from SB to SS [96], [98]. AQP3 is an aquaglyceroporin that transports water and glycerol. AQP3 is for example involved in the transport of glycerol to phospholipase D2, which can synthesize bioactive phosphatidylglycerol. Manipulation of phosphatidylglycerol has been reported to block keratinocyte proliferation and differentiation [96], [97]. However, studies examining the function of AQP3 have had contradictory results and therefore the specific role of AQP3 in the regulation of keratinocyte proliferation and differentiation remains unclear. In that respect, we would conduct an experiment in which keratinocyte cell lines are transfected with AQP3. We suggest using a spontaneously transformed immortal keratinocyte cell line from human skin known as the HaCaT cell line [99]. The HaCaT cell line is an excellent model for analyzing keratinocyte differentiation due to its high capacity to differentiate and proliferate *in vitro* [99], [100]. By comparing a HaCaT cell line transfected with AQP3 or another aquaporin, AQP5, to a knockout cell line, we may be able to determine how aquaporins affect proliferation and differentiation. These cells would be grown in a medium for 6-48 hours before cell growth measurement [101]. A study by Nakahigashi et al. examined whether overexpression of AQP3 would enhance keratinocyte proliferation. In this study, normal human keratinocytes were transfected with either an empty vector or a plasmid expressing human AQP3. The findings showed increased proliferation using a plasmid expressing this aquaporin compared to the cells transfected with an empty vector. To measure cell growth, Nakahigashi et al. performed the modified MTT assay, which will be described in greater detail in the next section [101]. We

expect increased keratinocyte proliferation for the HaCaT cell lines exposed to human AQP3-expressing plasmids.

Modified MTT Assay for Cell Growth Measurement

To determine cell growth of the HaCaT cell line, we would employ the modified MTT assay, illustrated in *figure 7*. Before performing this assay, control and transfected cell lines are plated in a well plate and incubated for at least 24 hours. After incubating, the MTT reagent, which is dissolved in PBS, is added to the cells. The MTT reagent is light sensitive, and therefore it is preferred to do the reaction in dark settings. The plate is then incubated for four hours after the addition of the MTT reagent. The procedure is followed by adding a solubilization solution like dimethyl sulfoxide (DMSO) and incubated again. The absorbance can now be monitored between 550 and 600 nm, where MTT dye absorbs. A curve can be made with the absorbance on one axis and the cell number on the other axis. Based on this, we can analyze the cell growth for the HaCaT cell line [102]–[104].

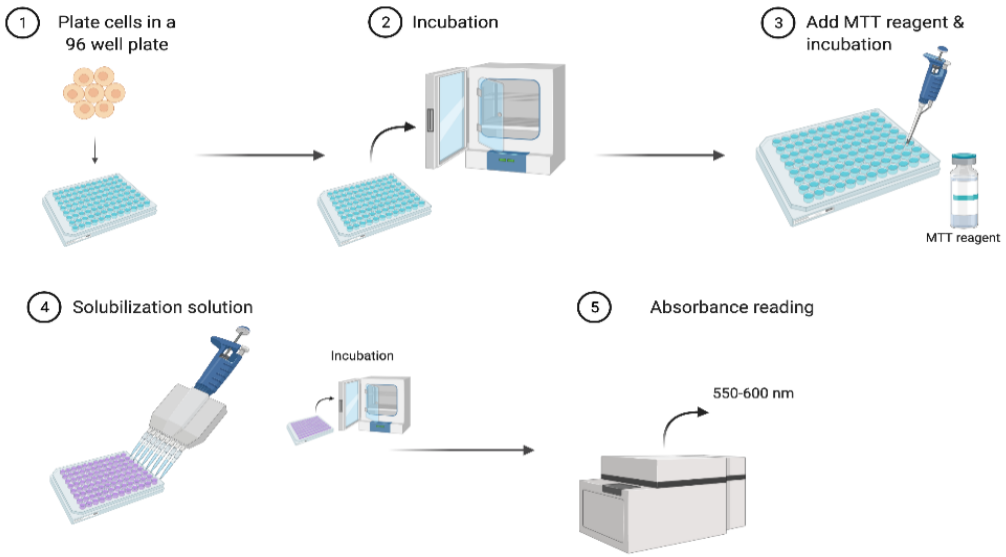

Figure 7. *Flowchart of the MTT assay procedure. HaCaT cells are plated in a 96-well plate and incubated for 24 hours. After the incubation, the MTT reagent is added to the well plate and incubated again. The solubilization solution is added and followed by incubation. The absorbance can be read between 550-600 nm. Created in BioRender.com.*

The other aquaporin, AQP5, is most likely involved in sweat secretion and may regulate the balance between the proliferation and differentiation of keratinocytes. It has been reported that the expression of AQP5 is decreased in the eccrine sweat glands of HS patients [101]. This

leads us to the hypothesis that AQP3 is also downregulated in HS skin, and it would therefore be interesting to measure the expression of this aquaporin in HS skin biopsies. In a study by Boury-Jamat et al. the expression and function of AQP3 was determined in different skin cells, including keratinocytes. One of the methods used in this study was the one-step reverse transcription polymerase chain reaction (RT-PCR) [96]. Therefore, we consider one-step RT-PCR as a relevant method in determining the expression of AQP3 in HS skin. Before conducting this experiment, we would obtain skin biopsies of HS lesional, non-lesional, and healthy skin (control). Afterwards, we would extract and purify the total amount of RNA from the biopsies.

Extraction of RNA from *Ex Vivo* Skin

Total RNA can be purified from *ex vivo* skin by TRIzol extraction. TRIzol is a solution consisting of phenol and guanidine isothiocyanate. Phenol extracts nucleic acids and proteins, while guanidine isothiocyanate denatures proteins. Before the researcher can proceed with TRIzol extraction, they must ensure an environment free from RNase, which is an RNA-degrading enzyme [105]. This is a general rule when working with RNA [106]. Then the researcher can proceed with the first step in TRIzol extraction, which is tissue homogenization. Tissue homogenization lyses tissue cells, thereby releasing their contents [107]. This can be achieved by freezing and subsequently powdering the tissue on dry ice. Afterwards, TRIzol reagent is added, and the cells are collected by centrifugation. Then chloroform is added, and the solution is subsequently vortexed and centrifuged. This produces two phases in which the upper clear phase contains RNA. The upper phase is transferred to a fresh tube and isopropanol is added. This is followed by vortexing and centrifuging the solution, which results in precipitation of RNA. The supernatant is discarded, and the pellet is resuspended in a buffer. Thereafter, NaOAc is added, and RNA is re-extracted with phenol. The RNA is then precipitated with ethanol. Lastly, the purified RNA is stored at -20°C. The RNA is either stored in ethanol or recovered by centrifugation and resuspended in RNase-free water [108].

One-Step Reverse Transcription (Quantitative) Polymerase Chain Reaction (RT-(q)PCR)

In one-step RT-(q)PCR target RNA is reverse transcribed into complementary DNA (cDNA), which is then amplified by PCR, see *figure 8*. One-step RT-(q)PCR differs from two-step RT-(q)PCR in that all the components are added to one reaction tube instead of two. The procedure

of RT-PCR may vary depending on the kit; therefore, we will be referring to the protocol; SuperScript One-Step RT-PCR with Platinum *Taq* DNA Polymerase (Invitrogen) [109]. First, the researcher must add all the components of RT-PCR to one reaction tube. These components include buffer, Mg^{2+}, target RNA, reverse transcriptase, gene-specific primers, deoxyribonucleoside triphosphates (dNTPs) and the thermostable DNA polymerase; *Taq* polymerase. Furthermore, the *Taq* polymerase is complexed with an antibody. Then the reaction tube is transferred to a pre-heated thermal cycler set at 45-55°C. This enables complementary primers to anneal to the target RNA, which is subsequently converted into cDNA by the enzyme reverse transcriptase. Reverse transcriptase synthesizes cDNA using dNTPs such as adenine and cytosine. If done efficiently, RT can be achieved in 15-30 minutes. Before PCR can begin, the temperature must be raised to 94°C for two minutes to reduce non-specific DNA amplification [110]. This denatures the *Taq* antibody, thereby activating *Taq* polymerase. Likewise, the RNA/cDNA hybrids are denatured, and the reverse transcriptase is inactivated by the high temperature. Afterwards, amplification can begin, which can be done by standard PCR or quantitative PCR (qPCR). In both methods, the first step is raising the temperature to 94°C for 15 seconds. Then the solution is cooled down to 55-60°C for 30 seconds in order for complementary primers to anneal to the cDNA. Subsequently, the temperature is held constant at 68-72°C, while *Taq* polymerase utilizes dNTPs to synthesize new complementary DNA strands. The extension time varies with the size of the PCR product; however, it takes approximately one minute per one kb of PCR product. This marks the end of the first cycle out of 35-40 cycles in which the amount of cDNA is doubled after each cycle. Optionally, a final extension can be performed at 72°C for five to ten minutes [109]. In standard PCR, the PCR product is stained with ethidium bromide, electrophoresed on an agarose gel and photographed under fluorescent UV light [111]. Before performing RT-PCR, the researcher determines the expected length of the PCR product in order to identify the target cDNA on the agarose gel. On the other hand, qPCR detects a fluorescent signal during amplification. The signal is provided by fluorescent probes attached to the primers or double-stranded DNA-binding dyes such as SYBR Green. The fluorescence increases proportionally with the PCR product during amplification and a camera records these changes in fluorescence [110], [112].

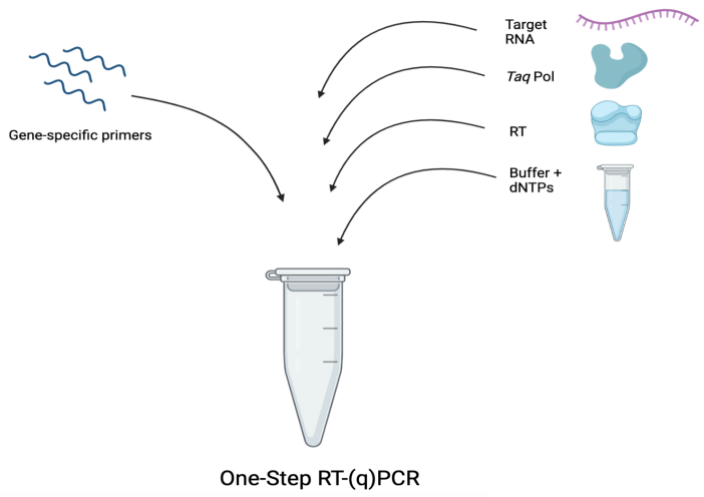

Figure 8. *Principle of one-step RT-(q)PCR. The reagents of RT and (q)PCR are added to one reaction tube. These reagents include target RNA, buffer, dNTPs, Taq polymerase, reverse transcriptase, and gene-specific primers. Created in BioRender.com*

Gene Expression of AQP3 and AQP5

As previously mentioned, we would need to obtain skin biopsies from healthy individuals and patients with HS to measure the expression of AQP3. AQP3 mRNA from these biopsies would be purified by TRIzol extraction, as described above. Then we would design a primer that is specific for AQP3 mRNA, which would be used in RT-(q)PCR. After completing these methods, we would compare the expression of AQP3 in HS lesional, non-lesional and healthy skin by analyzing the fluorescent signals emitted by cDNA on the agarose gel or during amplification. To confirm the downregulation of AQP5 in patients with HS, we would employ the same techniques. In connection with the regulation of aquaporins, it is relevant to look at whether molecules that block or promote enzyme function can affect the pathogenesis of HS. We have selected the following inhibitors and activators based on current literature and research in the treatment of HS, including aquaporins.

Inhibition of Histone Deacetylase-3 (HDAC3)

AQP3 is inhibited by the enzyme histone deacetylase-3 (HDAC3), meaning the inhibition of HDAC3 could potentially increase the expression of AQP3. HDACs allow the tight wrapping of DNA around histones by removing acetyl groups from the lysine residues at the N-terminal region of these proteins. HDAC inhibitors (HDACIs) suppress the activity of HDACs by occupying the catalytic core of their Zn^{2+}-containing active site. Several HDAC3 inhibitors are currently under investigation, however, some are approved by the Food and Drug

Administration (FDA) in the US. One example is vorinostat, a hydroxamic acid derivative that is used to treat cutaneous T-cell lymphoma [113].

PPARγ Activator

It has also been observed that activation of the peroxisome proliferator-activated receptors (PPARγ) increases the AQP3 mRNA expression in both differentiated and undifferentiated human keratinocytes. PPARγ regulates the expression of many essential aspects of keratinocyte metabolism and thus might be important to investigate to improve the treatment of skin disorders such as HS [114].

Tanshinol

A study from 2018 has shown tanshinol to increase the expression of AQP5, which was measured by RT-qPCR and Western blotting [115]. Tanshinol is used in the treatment of cardiovascular disease [116]. A study by Jianfeng et al. suggests that tanshinol may be involved in increasing the expression of AQP5 by downregulating inflammatory cytokines. The reduced levels of cytokines might indicate that tanshinol protects damaged tissue and inhibits systemic inflammation by upregulating AQP5 expression [115].

Target 2: Matrix Metalloproteinases

As previously mentioned, MMPs play several important roles in different physiological processes, and are thus relevant to investigate in relation to HS [63], [117]. Therefore, we have chosen to study MMPs as potential drug targets for the treatment of HS. Since elevated levels of particularly MMP-2, MMP-8, and MMP-9, have been observed in HS lesions, we have chosen to focus on these specific isoforms [74], [75], [118], [119]. Similar trends regarding elevated levels of specific MMPs are observed in psoriasis skin [120]–[123]. MMPs are activated as part of the inflammatory immune response, meaning that these targets are prominent in the later stages of HS. MMPs can be presented in two forms, latent (zymogen) and activated. The activated form can be present in a complex with tissue inhibitors of metalloproteinases (TIMPs) and other natural inhibitors [124]. TIMPs are essential in order to maintain the balance between ECM remodeling and breakdown, and an imbalance seems to contribute to a number of diseases [67], [125], [126]. MMPs have several domains in common, including the pro-domain, the catalytic domain and the hemopexin domain that is attached to the C terminus. MMPs are produced in the latent form and become active when the pro-domain

is removed by proteolysis [64], [65], [127]. The catalytic domain contains two zinc ions (Zn^{2+}) and is necessary for structural integrity as well as the proteolytic activity. Lastly, the hemopexin domain contributes to the recognition and binding of substrates and interaction with endogenous inhibitors [63]. The substrates encompass tyrosine kinase receptors, peptide growth factors, cell-adhesion molecules, chemokines, and cytokines along with unrelated proteases and other MMPs [64], [65]. As already stated, MMPs can be divided into six groups. MMP-8 belongs to the collagenase subgroup and is found to be upregulated in HS lesions [75], [76]. Collagenases degrade extracellular matrix components as well as cleave several types of interstitial collagens, including type I, II and III. However, MMP-8 exhibits higher proteolytic activity against collagen type I [63], [128]. In contrast, MMP-2 and MMP-9 belong to the group of MMPs called gelatinases and differ from MPP-8 and other MMPs in their substrate specificity and structure. However, gelatinases possess the same basic structure as other MMPs and, in addition, contain fibronectin repeats in the catalytic domain [63], [129]. The fibronectin domain is located in the zinc-binding domain (metalloproteinase domain) and the active site of the enzyme. It consists of three repeats of the fibronectin type II-motif, and can among others, bind to collagen type-I and VI [63], [130]–[132]. The fibronectin domain also possesses high affinity binding to gelatin and is involved in proteolytic activity against gelatin [133], [134]. Dysregulation and overexpression of MMP-9 seems to be associated with some-diseases, thus emphasizing the importance of examining its inhibition and regulation, in relation to HS. Due to the structural similarities between MMP-2 and MMP-9, the inhibition of MMP-9 is also likely to result in the inhibition of MMP-2 and vice versa [123], [129].

MMP Inhibitors (MMPIs)

Over time, different generations of MMPIs have been developed, each with its own characteristics. These generations include the first generation (hydroxamate-based inhibitors), the second generation (non-hydroxamate-based inhibitors), the third generation (catalytic domain (non-zinc binding) inhibitors) and lastly the fourth generation (allosteric and exosite inhibitors) [124], [135]. Unfortunately, initial clinical trials as well as animal models of MMPIs have been disappointing and have thus led to a negative view of MMPs as therapeutic targets [63], [124], [135]. However, a better understanding of the pharmacokinetic properties of the inhibitors as well as a better insight into the biology of MMPs suggest that the initial trials have been performed prematurely and have subsequently helped to restore interest in inhibiting MMPs as a therapeutic target [63], [124]. In particular, the latest generation of MMPIs shows

promising results and possesses improved pharmacokinetics as well as preferable selectivities [124], [135], [136]. Additionally, ongoing research focusing on protein-engineered inhibitors and antibody-based inhibitors, has further improved the MMPI design. Recently, newer antibody-based inhibitors have been developed that have shown high affinity as well as truly selective properties [124], [126], [135]. Advantages of using anti-MMP antibodies include their ability to be engineered and designed to selectively inhibit the individual isoforms of MMPs, such as MMP-2, MMP-8, and MMP-9. In fact, antibodies successfully targeting MMP-9 have already been studied [67], [125], [137]. Furthermore, a research group, Sela-Passwell et al. have managed to produce antibody inhibitors with ability to bind MMP-2 and MMP-9. These inhibitors are comparable to the endogenous TIMPs. In addition, the antibody inhibitors passed multiple clinical phases [126]. Based on the current knowledge, we would recommend testing an antibody-based inhibitor in our experimental design. The antibody inhibitor should be designed to bind to the catalytic site of MMP-2, MMP-8, and MMP-9, since this domain is crucial for proteolytic activity [63], [138], [139].

Human Skin Biopsies

Human skin biopsies are relevant in pre-clinical trials to aid development of new drugs as the failure rate may increase when animal models are used. Human skin biopsies used in clinical trials are often very small, around 3-6 mm in diameter [17], [74], [140]. Skin biopsies are relevant in the research of disease pathways because they are highly complex. Skin biopsies can be obtained in different ways, surgically or by the punch method among others. Using the punch method, the biopsies are typically small, whereas biopsies obtained surgically, are larger. All types of skin biopsies can be used for *ex vivo* culturing. That means that they can be kept alive, which makes them suitable for investigation of the effectiveness of a potential drug [140]. Skin biopsies can be obtained with different purposes, but to investigate the inflammatory response in HS, skin biopsies surgically removed from healthy individuals and from patients with HS, are frequently used and compared [17]. For further investigation of the disease, biopsies from HS lesions and from perilesional HS skin are typically used [74].

Ex Vivo Skin Cultures

Ex vivo skin cultures can be used for a wide range of genetic studies, including gene expression and protein release. In general, human skin models are useful for drug development, and especially *ex vivo* skin models are extremely disease-relevant [140]. It is important to be aware that *ex vivo* skin cannot be cultured indefinitely, as they in general can only be kept "alive" in culture for seven days. The durability of the biopsies, however, depends on several factors, where the chosen size of the biopsies and the media change regime have a great influence. It is optimal to complete the experiments and analysis within four days, as the epithelium and cells change over time [17], [140]. There are many different methods for culturing *ex vivo* skin, however, we have chosen to focus on three different *ex vivo* skin culture models; liquid-submersion, air-liquid interface and the perfusion bioreactor skin culture model, all illustrated in *figure 9* [17], [140]. In liquid submersion culture, the entire three-dimensional tissue is immersed in the culture media, thus reminiscent of traditional cell culture. In contrast, the air-liquid interface mimics the original tissue environment to a greater extent, given that air is available to the apical tissue surface, while the basal surface is submerged in the media. The perfusion bioreactor permits continuous perfusion of nutrients/oxygen, and thus mimics the real circulatory system [17], [140]. The three aforementioned *ex vivo* skin culture methods have their advantages as well as disadvantages, but all allow an effective characterization of tissues [17], [140]. Furthermore, they can help evaluate potential drug targets against various skin diseases, such as HS [17], [140].

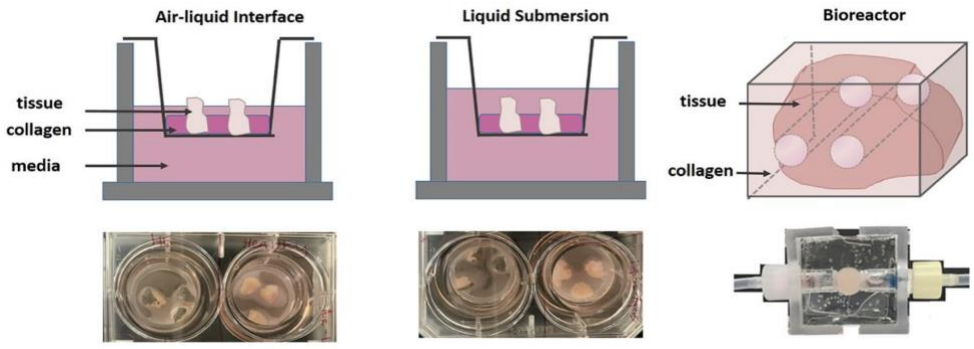

Figure 9. *Overview of Platforms for Air–liquid interface, Liquid submersion culture and Perfusion bioreactor culture* [17].

Electrophoresis

Gel electrophoresis is a technique used to separate DNA fragments, RNA or proteins based on their size and electrical charge. The separation is done by running a current through a gel containing the samples. DNA is negatively charged, therefore, when exposed to an electrical charge, the DNA will travel towards positively charged electrodes. The fragments migrate according to their size, with the smallest molecules traveling through the gel quickest. This method enables us to determine the amount of DNA fragments in our sample, as well as their molecular mass [141], [142].

Gelatin Zymography

Gelatin zymography is a non-quantitative technique, which uses the ability of gelatinases, such as MMP-2 and MMP-9, to hydrolyze denatured gelatin. Thus the presence of gelatinase in samples such as *ex vivo* skin cultures can be determined [143], [144]. As mentioned previously, MMPs are expressed as latent proenzymes (pro-MMPs) that are activated through a conformational change, triggered by proteolytic cleavage [64], [65], [127]. Gelatin zymography uses sodium dodecyl sulfate (SDS)-polyacrylamide gels impregnated with gelatin, as substrate. SDS ensures that the proteins are separated based on their molecular mass, enabling us to measure the amount of MMP-2 and MMP-9 in the samples individually [134]. The use of electrophoresis and SDS denatures the MMPs, resulting in a dissociation of the pro-domain from the active site. After partial renaturation and exposing the active site, the gelatinases can initiate the degradation of gelatin. The activity of the MMPs is restored by incubating the gel in Triton X-100 followed by incubation at 37°C overnight in a buffer used to remove SDS from the gel. This enables the MMPs to hydrolyze gelatin. The gelatin zymogram is subsequently stained, and areas of hydrolyzed denatured gelatin appear as clear bands against a Coomassie Blue stained background. The molecular mass of active and latent gelatinases corresponds to the clear bands, showing up as white on *figure 10*. To assess the molecular size of the bands it is necessary to have prepared a pre-stained standard enzyme with which the samples can be compared [134]. Pro-MMP-2 and MMP-2 can be read as 72 and 68 kDa bands, respectively [145]. Pro-MMP-9 and MMP-9 have a higher molecular mass and are therefore found closer to the negative pole of the gel. Pro-MMP-9 and MMP-9 can be read at 92 kDa and 82 kDa, respectively [146], [147].

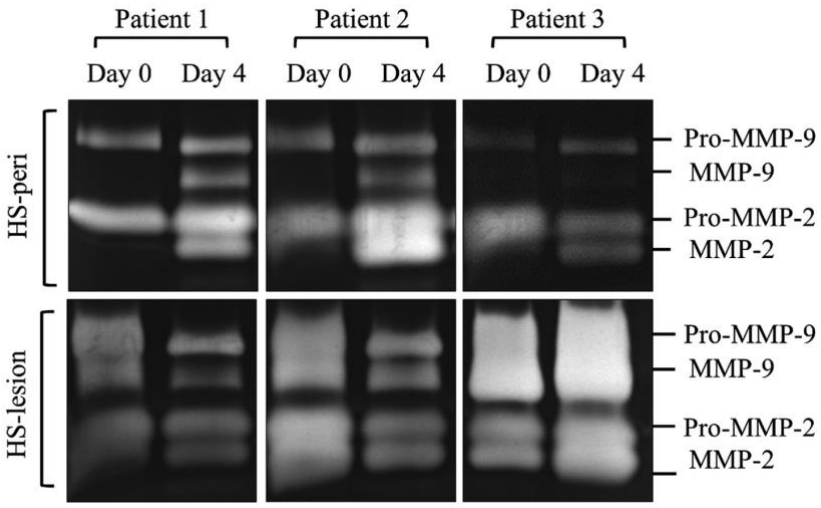

Figure 10. *Detection of MMP through gelatin zymography in HS skin from Sanchez et al. [74].*

It is important to note that during electrophoresis inhibitor-bound gelatinases are disassociated. Therefore, zymography does not distinguish between gelatinases that were free or inhibited prior to electrophoresis [134], [148]. If you wish to obtain the overall net gelatinase activity, additional protein activity assays are necessary to conduct [134].

Collagen Zymography

Collagen zymography is a technique, very similar to gelatin zymography. The main difference of the methods is that the substrate used in collagen zymography is collagen, instead of gelatin. The major principles of collagen- and gelatin zymography are the same and will for that reason not be described in further details. Collagen zymography is the preferred method for examining the presence of collagenases, such as MMP-8, and like gelatin zymography, this technique is able to identify both latent and active forms of collagenases [147], [149].

ELISA

Enzyme-linked immunosorbent assay (ELISA) method allows detection of antigens such as proteins, peptides, or hormones, even in low quantities and concentrations. This method uses specific antibodies in combination with an enzyme-mediated color change, to detect the presence of antigens [150]–[152]. During ELISA, the antigen is immobilized on a solid surface (normally in 96-well microliter plates) that bind antigens and antibodies passively [150], [153]. The immobilization of reactants to the microplate surface provides an easy way to separate bound material from non-bound [151], [153]. An antibody specifically designed for binding an antigen is attached to the bottom of the well. Then, the antigen is specifically detected by

another enzyme-coupled antibody, and when adding a colorless substrate, the enzyme produces a colorful product that can be measured [151]–[153]. There are three different types of ELISA; direct, indirect and sandwich ELISA, as illustrated in *figure 11*. In direct ELISA, the antigen is immobilized directly on the bottom of the plate. Then the enzyme-coupled antibody binds to the antigen. In indirect ELISA, the antigen is also immobilized directly on the bottom of the plate, but here a primary antibody binds the antigen, and a secondary enzyme-linked antibody binds the primary antibody. Nonetheless, sandwich ELISA is the most commonly used method, described in the section below [150]–[153].

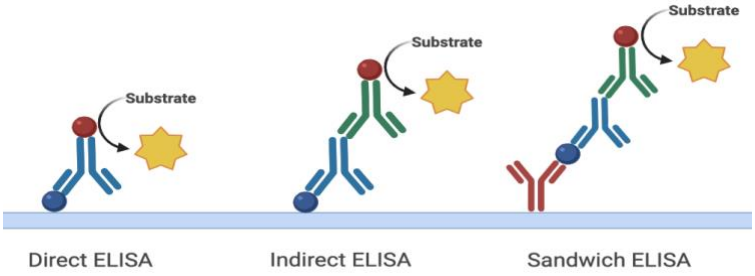

Figure 11. *Comparison of ELISA assays. Created in BioRender.com.*

Human MMP ELISA Kit

Human MMP (Hu MMP) can quantitatively be detected using sandwich ELISA. The amount of target between an antibody-pair can be measured using the Hu MMP solid-phase sandwich ELISA. First, a target-specific primary antibody is added to the bottom of the well plate after the samples of interest are added to the wells as well. Then, the target antigen in the solutions binds the immobilized captured antibody. Afterwards a detector antibody is added to the wells and the sandwich is formed by binding to the target antigen. A secondary enzyme-linked antibody, which will bind to the detector, is also added. Lastly a substrate solution is added to the wells producing a measurable signal. The substrate solution reacts with the enzyme-linked antibody-target-complex. The intensity of the signal directly correlates to the concentration of MMPs present in the original sample [153]–[156] . As we focus on MMP-2, MMP-8, and MMP-9, respectively, we propose using a Hu MMP Kit designed for each MMP in the experimental design.

Experimental Design

As mentioned earlier, upregulation of MMPs may be linked to multiple complications. In that respect, we suggest conducting an experiment that determines the protein activity of MMP-2, MMP-8, and MMP-9 in HS skin and skin from healthy controls using *ex vivo* model cultures. We also propose testing whether potential antibody-based inhibitors are able to bind to MMP-2, MMP-8, and MMP-9. The inhibition of the aforementioned MMPs could be a potential treatment for HS. To investigate the protein amount of MMP-2, MMP-8, and MMP-9, *ex vivo* HS skin as well as healthy controls must be cultured for a maximum of four days, since the tissue continuously changes [17], [140]. For comparability purposes, it is crucial that the HS patients have reached the same Hurley stage and that the skin biopsies are of similar size. Three separate skin biopsies should be taken from each HS patient, including lesional skin, perilesional skin, and uninvolved skin (10 cm away from the lesion). The severity of the lesions needs to be considered as well, more precisely whether they are fistulas, scar tissue, abscesses, nodules etc., as the presence of MMPs may vary depending on the severity of the lesions [5].

Analysis of MMPs

To measure the amount of MMP-2 and MMP-9, we have chosen the gelatin zymography technique described more in depth in the sections above. Since MMP-8 is a collagenase, we suggest using collagen zymography to investigate this MMP [63]. To determine whether there is a significant difference between the amount of MMP-2, MMP-8, and MMP-9 in HS skin and healthy controls, it is necessary to perform some statistical tests. In this regard, we would recommend a significance level of 0.05. To obtain the quantitative amount of MMP-2, MMP-8, and MMP-9, we suggest using Hu MMP ELISA Kit on the supernatants from the cell cultures. We have described the basic principles of Hu MMP ELISA in the sections above. For our experiment it is necessary to perform three separate Hu MMP ELISA assays, however, the basic techniques are the same for the three assays [154]–[156]. Another way we can apply ELISA is to investigate whether potential inhibitors can bind to our targets. We would recommend testing an antibody-based inhibitor with direct ELISA. These assays must be performed separately, one for each MMP. If it turns out that the potential inhibitors are capable of binding MMP-2, MMP-8, and MMP-9, then subsequent tests can be performed to determine whether inhibition of MMPs has a promising effect on the severity of the disease [150], [153]. Nonetheless, the efficacy of the inhibitors is not the focus of this experiment and will not be commented on further.

Discussion

Potential Drug Targets Compared with Current Treatment

Current treatment options offered to HS patients have varying response rates, however, these therapies are in general not very effective. Furthermore, long-term treatment with antibiotics may result in bacteria developing antibiotic resistance [157]. Likewise, the patient can develop resistance towards other drugs that are used to treat HS. In the worst-case scenario, it will no longer be possible to treat a patient with most of the current therapies, excluding laser treatment and surgery. This is very problematic and emphasizes the need for new therapies. However, developing effective treatments for HS seems to be very difficult, which is partly due to the fact that several factors affect the disease [5], [12].

Human Skin Biopsies Versus Animal Models

There are several advantages considering the use of human skin biopsies in trials examining the development of new drugs. One of the benefits of using human skin tissues over animal models is the lack of molecular mismatches due to genetic species variation. Without these mismatches, it may be easier to predict the response to a new drug. Therefore, potential drugs that fail in preclinical trials using skin biopsies from animals may be approved when human skin is used instead. The relevant biologic systems and pathways can also be investigated more properly when healthy human tissue is compared to diseased tissue. Economical aspects should also be considered when discussing the use of human skin biopsies over animal models. By using ex vivo skin, the process of developing a new drug may be faster and thus more cost-effective. However, human skin biopsies can be difficult to obtain, whereas skin biopsies from animals in general are easily obtained. To date, there are no animal models of HS, which contributes to the lack of knowledge that exists in the pathogenesis of HS. A way to contribute to the understanding of HS is finding new targets. Since HS progresses at different stages, we have chosen targets that may be relevant at both the early and the late stages [140].

Target 1: Aquaporins

Aquaporins were selected as new potential targets due to their roles in the proliferation and differentiation of keratinocytes. As previously mentioned, abnormal proliferation and differentiation may lead to hyperkeratosis, one of the first events in developing HS lesions. Nonetheless, the functions of AQP3 and AQP5 are not fully understood, and thus far the importance of these targets in HS is not supported by sufficient evidence [97]. Nonetheless, we hypothesized that both aquaporins are downregulated in HS skin. In that respect, we proposed potential drugs that may increase the expression of AQP3 and AQP5. These drugs include the HDAC3 inhibitor vorinostat, activation of PPARγ, and tanshinol [113]–[115]. However, these drugs are included in trials concerning other diseases, such as sepsis and therefore the results cannot be directly transferred to the treatment of HS [115]. Furthermore, we do not know how the drugs will affect other factors contributing to HS. We cannot even be certain that upregulation of AQP3 and AQP5 is beneficial for HS patients. Therefore, more studies focusing on aquaporins should be performed, in order to offer better treatment options to HS patients in the future.

Target 2: Matrix Metalloproteinases

As already stated, MMPs are prominent in the later stages of HS since they are activated as part of the inflammatory immune response [158]. Due to their ability to cleave ECM components, it is reasonable to believe that the observed overexpression of MMPs in HS skin contributes to the pathogenesis and severity of the disease [63], [67], [158]. Therefore, it also emphasizes MMPs as potential therapeutic targets [63], [67]. In addition, it gives rise to the investigation of MMPIs as new treatment options for HS [124], [135].

Anti-MMP Antibodies

As previously described, some antibodies directed against MMP have been successfully investigated, and Sela-Passwell et al. have managed to produce inhibitory antibodies with similar binding mechanisms to MMP-2 and MMP-9 as the endogenous TIMPs [67], [125], [126], [137]. These are thus uplifting discoveries and emphasize the importance of continuing research into antibody-based inhibitors targeting MMPs. Assuming that dysregulation between MMPs and TIMPs may play a crucial role in the pathogenesis of HS, it is not improbable that antibody-based inhibitors capable of mimicking TIMPs may be introduced as an extension to

the current treatment options for HS patients in the future [124], [125]. Nonetheless, a new inhibitor must undergo a series of clinical trials before it can be implemented in medical practice [63], [126]. It is important not to disregard the potential side effects as well as the risk of off-targets that may occur using inhibitors. Therefore, it is also necessary to analyze these possible side effects and assess whether they are acceptable or not. Unfortunately, over time, many of the inhibitors have failed clinical trials, however, there still appears to be a great potential to be gained in this field [124]–[126], [135]. Antibody-based treatments are also not new occurrences regarding the treatment of HS. As already stated, treatment with the anti-TNF-α antibody, ADA, has shown promising results, thereby suggesting antibody-based MMP inhibitors as realistic therapeutic targets in the future [82].

Advantages and Disadvantages of the Methods

RT-(q)PCR

To study the gene expression of AQP3 and AQP5 in HS skin, we chose RT-(q)PCR as our method. This choice was inspired by multiple studies examining the expression of these aquaporins in normal skin. Nonetheless, this method has its advantages and disadvantages. As previously mentioned, either a one-step or two-step reaction can be utilized when performing RT-(q)PCR. The advantages of using a one-step reaction include a reduced risk of DNA contamination, only having one reaction tube, fewer reagents, and the fact that all the cDNA synthesized during RT can be used for (q)PCR. On the other hand, excessive cDNA produced during a two-step reaction can be stored for later use. Furthermore, the reaction conditions for RT and (q)PCR can be optimized when a two-step reaction is performed because reverse transcriptase and *Taq* polymerase act in different reaction tubes. Thereby, the yield of cDNA may increase. In the analysis, we also described how we would either conduct standard PCR or qPCR. In standard PCR, the number of PCR products is detected at the plateau phase, which occurs after 30-45 cycles, so the number of reagents may not be sufficient at this point. By contrast, qPCR detects the number of PCR products during the exponential phase of the amplification and as a result is more efficient at quantifying cDNA compared to standard PCR. However, qPCR is more sensitive than standard PCR, which makes it incredibly important that the RNA template is not contaminated with DNA during RT. Nevertheless, this can be avoided by adding DNase when RT is performed. Another advantage of qPCR is the fact that it is less

time-consuming than standard PCR because the use and subsequent analysis of an agarose gel is avoided [159], [160].

Ex Vivo Skin Culture

Use of ex vivo skin has great potential for various genetic studies, and especially ex vivo skin cultures can be highly relevant for diseases such as HS. In this regard, it is crucial to consider the type of media in which the ex vivo skin is cultured. In the experimental design regarding MMPs, we have introduced three different methods for culturing biopsies: liquid-submersion, air-liquid interface, and the perfusion bioreactor skin culture model. Each skin culture method has its related advantages as well as disadvantages. For example, to investigate new potential drugs, the biopsies must be in a culture that mimics the native environment as much as possible in order to predict the actual response [17], [140]. Since the air-liquid interface skin culture is the model that provides the most realistic conditions (due to the exposure of air to the epidermis), one can argue that it is the optimal skin culture model. On the other hand, it can also be argued that the perfusion bioreactor is even better as this method permits continuous perfusion of nutrients/oxygen and thus mimics the real circulatory system [17], [140]. However, the experiment does not include the investigation of drug response, and thus one can argue that the choice between these three skin culture methods is not relevant. Furthermore, it is worth mentioning that all three *ex vivo* skin culture methods allow an effective characterization of tissues, and thus provide unique opportunities to investigate the pathogenesis of HS in general [17], [140].

Zymography

We have chosen methods that are commonly used in collaboration or separately when studying the amount of MMP-2, MMP-8, and MMP-9 as potential drug targets for HS and other diseases. When using zymography, the advantages and disadvantages of this method should be considered. Since there is no distinction between inactive and active forms of MMPs during zymography, the validity of the results is compromised [134], [148]. In our experiment, the gelatin zymography aims to detect the presence of MMP-2 and MMP-9 in *ex vivo* skin cultures. MMP-8 is, as mentioned, also capable of degrading gelatin and could therefore interfere with the presence of MMP-2 and MMP-9. However, this interference will have minimal effect on the results since gelatin is not the preferred substrate of MMP8 [149], [161]. Instead, collagen is used as a substrate for detecting MMP-8 present in our *ex vivo* cultures. Zymography is a

sensitive technique, and attention to detail is needed before such a method can be conducted. For example, the amount of sample loaded onto the gel can affect the quality of the results. If the amount of tissue extract is too large, it may result in distorted bands. Nonetheless, the sensitivity of zymography makes it capable of measuring low levels of MMPs. Unfortunately, low levels of MMPs are often not detected because the ratio between MMPs and total protein amount in the crude sample is too low [161]. Therefore, the right sample to substrate ratio is needed for a successful zymography. Another disadvantage is the fact that only 35% of the MMP catalytic activity is restored during protein refolding. Thereby, the measured amount of pro-MMP in zymography may not represent the true biological presence of active MMPs in the *ex vivo* skin [161]. This uncertainty contributes to the dissociation of non-covalently bound MMP inhibitors during electrophoresis, which makes the measured MMP amount during zymography less accurate than the actual amount in our sample. The results should, therefore, rather be seen as a measure of the potential amount of MMPs. The many steps of zymography may create problems when standardizing the results. When having multiple samples and parameters the possibility of variation makes it difficult to create results and conditions that can easily be reproduced. The results are therefore qualitative, and it is recommended to use multiple assays for measuring the presence of MMPs in biological samples. However, when conducted with attention to detail, zymography is a reliable method for measuring the presence of MMPs, and is widely used for non-quantitative results on enzyme amounts in experimental and biological systems [152], [161].

ELISA

As mentioned earlier, multiple versions of ELISA exist such as the sandwich- and direct ELISA. In our impression, sandwich ELISA is the most used method for analyzing the presence of MMPs. Unlike zymography, ELISA can be used as a quantitative or qualitative method and is, therefore more accurate. Thereby, it is recommended to supplement zymography with ELISA when measuring the presence of MMPs. For successful results with ELISA, the selection of antibodies is crucial. As an example, a high binding affinity between the antibody and antigen reduces the ability of non-specific substance interference. Other advantages of sandwich ELISA include high sensitivity and high target accuracy, as it uses two antibodies for capture and detection. Additionally, different detection methods can be used with the same capture antibody. However, compared to other ELISA methods, sandwich ELISA requires

more optimization to identify antibody pairs and to ensure there is limited cross-reactivity between the capture and detection antibodies [153], [162].

Multiple Factors Influence the Disease

The search for more efficient therapies for patients suffering from HS, appears to go beyond the identification of a single gene or pathway. Even if a particular drug is discovered, approved, and used as treatment it may not benefit all HS patients as the disease seems to be multifactorial. Smoking and obesity display a pathological influence in relation to an abundance of epidermal cells and skin friction, thereby elevating the risk of developing HS [14], [49].

Conclusion

Based on this report, it can be concluded that HS is a debilitating autoinflammatory disease with a complex pathogenesis. Several factors appear to influence the disease, resulting in the lack of optimal treatment options as well as complicating the development of new drugs. After studying the current knowledge regarding the underlying mechanisms of HS, we have presented the potential drug targets, AQP3, AQP5, MMP-2, MMP-8, and MMP-9. These targets may be significant at either the early or the late stages of HS. Furthermore, we have suggested methods that might help examine the relevance of the potential targets in HS. Nonetheless, future research on the molecular basis of HS is needed to achieve successful treatment options for HS patients.

References

[1] S. Abranowicz and K. Watson, "What Is Hidradenitis Suppurativa? Symptoms and Treatment Options — Dermatologist Advice | Allure," *Skin-Care Guide*, 2020. https://www.allure.com/story/hidradenitis-suppurativa-acne-symptoms-causes-treatments (accessed May 19, 2022).

[2] T. Montero-Vilchez *et al.*, "The Burden of Hidradenitis Suppurativa Signs and Symptoms in Quality of Life: Systematic Review and Meta-Analysis," *Int. J. Environ. Res. Public Heal. 2021, Vol. 18, Page 6709*, vol. 18, no. 13, p. 6709, Jun. 2021, doi: 10.3390/IJERPH18136709.

[3] T. V. Nguyen, G. Damiani, L. A. V. Orenstein, I. Hamzavi, and G. B. Jemec, "Hidradenitis suppurativa: an update on epidemiology, phenotypes, diagnosis, pathogenesis, comorbidities and quality of life," *J. Eur. Acad. Dermatology Venereol.*, vol. 35, no. 1, pp. 50–61, Jan. 2021, doi: 10.1111/JDV.16677.

[4] L. A. V. Orenstein, T. V. Nguyen, G. Damiani, C. Sayed, G. B. E. Jemec, and I. Hamzavi, "Medical and Surgical Management of Hidradenitis Suppurativa: A Review of International Treatment Guidelines and Implementation in General Dermatology Practice," *Dermatology*, vol. 236, no. 5, pp. 393–412, Sep. 2020, doi: 10.1159/000507323.

[5] V. Amat-Samaranch, E. Agut-Busquet, E. Vilarrasa, and L. Puig, "New perspectives on the treatment of hidradenitis suppurativa:," *Sage Journals, Ther. Adv. Chronic Dis.*, vol. 12, Nov. 2021, doi: 10.1177/20406223211055920.

[6] A. Garg, J. Lavian, G. Lin, A. Strunk, and A. Alloo, "Incidence of hidradenitis suppurativa in the United States: A sex- and age-adjusted population analysis," *J. Am. Acad. Dermatol.*, vol. 77, no. 1, pp. 118–122, Jul. 2017, doi: 10.1016/J.JAAD.2017.02.005.

[7] M. Daxhelet *et al.*, "Proposed Definitions of Typical Lesions in Hidradenitis Suppurativa," *Dermatology*, vol. 236, no. 5, pp. 431–438, Sep. 2020, doi: 10.1159/000507348.

[8] Ballard Kimberly and Shuman Victoria L, "Hidradenitis Suppurativa - StatPearls - NCBI Bookshelf, NBK534867," *StatPearls*, 2022. https://www.ncbi.nlm.nih.gov/books/NBK534867/ (accessed May 16, 2022).

[9] H. Alsadhan, A. I. Alfawzan, A. Yaqoub, A. Almoneef, and M. Almohideb, "Hidradenitis Suppurativa: Estimated Prevalence, Clinical Features, and Risk Factors in Riyadh, Saudi Arabia," *Cureus*, vol. 14, no. 3, Mar. 2022, doi: 10.7759/CUREUS.23029.

[10] A. Garg, J. S. Kirby, J. Lavian, G. Lin, and A. Strunk, "Sex- and Age-Adjusted Population Analysis of Prevalence Estimates for Hidradenitis Suppurativa in the United States," *JAMA Dermatology*, vol. 153, no. 8, pp. 760–764, Aug. 2017, doi: 10.1001/JAMADERMATOL.2017.0201.

[11] S. W. Jiang, M. J. Whitley, P. Mariottoni, T. Jaleel, and A. S. MacLeod, "Hidradenitis Suppurativa: Host-Microbe and Immune Pathogenesis Underlie Important Future Directions," *JID Innov.*, vol. 1, no. 1, p. 100001, Mar. 2021, doi: 10.1016/J.XJIDI.2021.100001.

[12] R. Sabat, G. B. E. Jemec, Ł. Matusiak, A. B. Kimball, E. Prens, and K. Wolk, "Hidradenitis suppurativa," *Nat. Rev. Dis. Prim.*, vol. 6, no. 1, Dec. 2020, doi: 10.1038/S41572-020-0149-1.

[13] F. Canoui-Poitrine *et al.*, "Identification of Three Hidradenitis Suppurativa Phenotypes: Latent Class Analysis of a Cross-Sectional Study," *J. Invest. Dermatol.*, vol. 133, no. 6, pp. 1506–1511, Jun. 2013, doi: 10.1038/JID.2012.472.

[14] K. Wolk, O. Join-Lambert, and R. Sabat, "Aetiology and pathogenesis of hidradenitis suppurativa," *Br.*

J. Dermatol., vol. 183, no. 6, pp. 999–1010, Dec. 2020, doi: 10.1111/BJD.19556.

[15] M. O. Machado *et al.*, "Depression and Anxiety in Adults With Hidradenitis Suppurativa: A Systematic Review and Meta-analysis," *JAMA Dermatology*, vol. 155, no. 8, pp. 939–945, Aug. 2019, doi: 10.1001/JAMADERMATOL.2019.0759.

[16] L. Thorlacius, A. D. Cohen, G. H. Gislason, G. B. E. Jemec, and A. Egeberg, "Increased Suicide Risk in Patients with Hidradenitis Suppurativa," *J. Invest. Dermatol.*, vol. 138, no. 1, pp. 52–57, Jan. 2018, doi: 10.1016/J.JID.2017.09.008.

[17] K. F. Goliwas *et al.*, "Ex Vivo Culture Models of Hidradenitis Suppurativa for Defining Molecular Pathogenesis and Treatment Efficacy of Novel Drugs," *Inflammation*, vol. 45, no. 3, pp. 1388–1401, Mar. 2022, doi: 10.1007/S10753-022-01629-W/FIGURES/7.

[18] S. Hillion *et al.*, "The Innate Part of the Adaptive Immune System," *Clin. Rev. Allergy Immunol.*, vol. 58, no. 2, pp. 151–154, Apr. 2020, doi: 10.1007/S12016-019-08740-1.

[19] R. V. Luckheeram, R. Zhou, A. D. Verma, and B. Xia, "$CD4^+$T cells: differentiation and functions," *Clin. Dev. Immunol.*, vol. 2012, 2012, doi: 10.1155/2012/925135.

[20] K. M. Yatim and F. G. Lakkis, "A Brief Journey through the Immune System," *Clin. J. Am. Soc. Nephrol.*, vol. 10, no. 7, p. 1274, Jul. 2015, doi: 10.2215/CJN.10031014.

[21] H. Haskell, "Introduction," *Dermatopathology*, pp. 1–8, Jan. 2010, doi: 10.1016/B978-0-443-06654-2.00018-4.

[22] D. G. W. Johnston, B. Kirby, and D. J. Tobin, "Hidradenitis suppurativa: A folliculotropic disease of innate immune barrier dysfunction?," *Exp. Dermatol.*, vol. 30, no. 10, pp. 1554–1568, Oct. 2021, doi: 10.1111/EXD.14451.

[23] W. Gaastra, "The innate and adaptive immune systems - NCBI Bookshelf," *National Library of Medicine, National Center for Biotechnology Information, Informedhealth.org*, 2020. https://www.ncbi.nlm.nih.gov/books/NBK279396/ (accessed May 16, 2022).

[24] S. McComb, A. Thiriot, B. Akache, L. Krishnan, and F. Stark, "Introduction to the Immune System," *Methods Mol. Biol.*, vol. 2024, pp. 1–24, 2019, doi: 10.1007/978-1-4939-9597-4_1.

[25] A. S. Byrd *et al.*, "Neutrophil extracellular traps, B cells, and type I interferons contribute to immune dysregulation in hidradenitis suppurativa," *Sci. Transl. Med.*, vol. 11, no. 508, Sep. 2019, doi: 10.1126/SCITRANSLMED.AAV5908.

[26] Alberts B, Johnson A, Lewis J, and et al., "The Adaptive Immune System - Molecular Biology of the Cell - NCBI Bookshelf," *New York: Garland Science*, 2002. https://www.ncbi.nlm.nih.gov/books/NBK21070/ (accessed May 16, 2022).

[27] J. Parkin and B. Cohen, "An overview of the immune system," *Lancet*, vol. 357, no. 9270, pp. 1777–1789, Jun. 2001, doi: 10.1016/S0140-6736(00)04904-7.

[28] "Det medfødte immunsystem." https://www.asmabashir.com/Det_medfodte_immunsystem.pdf (accessed May 20, 2022).

[29] M. A. Linterman and C. G. Vinuesa, "T Follicular Helper Cells During Immunity and Tolerance," *Prog. Mol. Biol. Transl. Sci.*, vol. 92, no. C, pp. 207–248, Jan. 2010, doi: 10.1016/S1877-1173(10)92009-7.

[30] F. H. Martini, M. J. Timmons, and R. B. Tallitsch, "The Integumentary System," in *Human Anatomy*, 8. Edition., Pearson Education, 2014, pp. 86–107.

[31] W. B. Bollag, L. Aitkens, J. White, and K. A. Hyndman, "Aquaporin-3 in the epidermis: More than skin deep," *Am. J. Physiol. - Cell Physiol.*, vol. 318, no. 6, pp. C1144–C1153, Jun. 2020, doi: 10.1152/AJPCELL.00075.2020/ASSET/IMAGES/LARGE/ZH00062086950003.JPEG.

[32] S. Agarwal and K. Krishnamurthy, "Histology, Skin - StatPearls - NCBI Bookshelf," in *Histology, Skin*, 2021, pp. 1–6.

[33] H. Yousef, M. Alhajj, and S. Sharma, "Anatomy, Skin (Integument), Epidermis," *StatPearls*, Nov. 2021, Accessed: May 19, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK470464/.

[34] J. . Marks and J. J. Miller, "Lookingbill and Marks' Principles of Dermatology E-Book," *Elsevier Heal. Sci.*, 2017, Accessed: May 17, 2022. [Online]. Available: https://books.google.co.id/books?id=37ZEDwAAQBAJ.

[35] C. H. Gibbons and R. Freeman, "Cutaneous Autonomic Innervation: Assessment by Skin Biopsy," *Prim. Auton. Nerv. Syst.*, pp. 413–417, Jan. 2012, doi: 10.1016/B978-0-12-386525-0.00085-8.

[36] J. Martel, J. Miao, and T. Badri, "Anatomy, Hair Follicle - StatPearls - NCBI Bookshelf," in *Anatomy, Hair Follicle*, 2021, pp. 1–6.

[37] W. D. Losquadro, "Anatomy of the Skin and the Pathogenesis of Nonmelanoma Skin Cancer," *Facial Plast. Surg. Clin. North Am.*, vol. 25, no. 3, pp. 283–289, Aug. 2017, doi: 10.1016/J.FSC.2017.03.001.

[38] E. Hoover, S. Aslam, and K. Krishnamurthy, "Physiology, Sebaceous Glands," *Natl. Libr. Med. Natl. Cener Biotechnol. Information,StatPearls*, Oct. 2021, Accessed: May 16, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK499819/.

[39] I. C. Gebeshuber and G. van Aken, "Editorial: Friction and Lubricants Related to Human Bodies," *Lubr. 2017, Vol. 5, Page 4*, vol. 5, no. 1, p. 4, Feb. 2017, doi: 10.3390/LUBRICANTS5010004.

[40] S. R. Goldburg, B. E. Strober, and M. J. Payette, "Hidradenitis suppurativa: Epidemiology, clinical presentation, and pathogenesis," *J. Am. Acad. Dermatol.*, vol. 82, no. 5, pp. 1045–1058, May 2020, doi: 10.1016/J.JAAD.2019.08.090.

[41] L. K. Hoffman, M. H. Ghias, and M. A. Lowes, "Pathophysiology of hidradenitis suppurativa," *Semin. Cutan. Med. Surg.*, vol. 36, no. 2, pp. 47–54, Jun. 2017, doi: 10.12788/J.SDER.2017.017.

[42] L. B. Baker, "Physiology of sweat gland function: The roles of sweating and sweat composition in human health," *https://doi.org/10.1080/23328940.2019.1632145*, vol. 6, no. 3, pp. 211–259, Jul. 2019, doi: 10.1080/23328940.2019.1632145.

[43] X. Zhang, Y. Li, H. Xu, and Y. W. Zhang, "The γ-secretase complex: From structure to function," *Front. Cell. Neurosci.*, vol. 8, no. DEC, p. 427, Dec. 2014, doi: 10.3389/FNCEL.2014.00427/BIBTEX.

[44] A. E. Pink, M. A. Simpson, N. Desai, R. C. Trembath, and J. N. W. Barker, "γ-Secretase mutations in hidradenitis suppurativa: new insights into disease pathogenesis," *J. Invest. Dermatol.*, vol. 133, no. 3, pp. 601–607, 2013, doi: 10.1038/JID.2012.372.

[45] T. Nomura, "Hidradenitis Suppurativa as a Potential Subtype of Autoinflammatory Keratinization Disease," *Front. Immunol.*, vol. 11, May 2020, doi: 10.3389/FIMMU.2020.00847.

[46] E. Scala *et al.*, "Hidradenitis Suppurativa: Where We Are and Where We Are Going," *Cells 2021, Vol. 10, Page 2094*, vol. 10, no. 8, p. 2094, Aug. 2021, doi: 10.3390/CELLS10082094.

[47] R. Gratton *et al.*, "Pleiotropic Role of Notch Signaling in Human Skin Diseases," *Int. J. Mol. Sci.*, vol. 21, no. 12, pp. 1–29, Jun. 2020, doi: 10.3390/IJMS21124214.

[48] D. A. Gold, V. J. Reeder, M. G. Mahan, and I. H. Hamzavi, "The prevalence of metabolic syndrome in patients with hidradenitis suppurativa," *J. Am. Acad. Dermatol.*, vol. 70, no. 4, pp. 699–703, 2014, doi: 10.1016/J.JAAD.2013.11.014.

[49] F. Deilhes, R. M. Rouquet, Y. Gall, C. Aquilina, C. Paul, and M. P. Konstantinou, "Profile of smoking dependency in hidradenitis suppurativa patients and smoking cessation outcomes," *J. Eur. Acad. Dermatol. Venereol.*, vol. 34, no. 12, pp. e790–e791, Dec. 2020, doi: 10.1111/JDV.16494.

[50] A. König, C. Lehmann, R. Rompel, and R. Happle, "Cigarette smoking as a triggering factor of hidradenitis suppurativa," *Dermatology*, vol. 198, no. 3, pp. 261–264, 1999, doi: 10.1159/000018126.

[51] A. M. R. Schrader, I. E. Deckers, H. H. Van Der Zee, J. Boer, and E. P. Prens, "Hidradenitis suppurativa: a retrospective study of 846 Dutch patients to identify factors associated with disease severity," *J. Am. Acad. Dermatol.*, vol. 71, no. 3, pp. 460–467, 2014, doi: 10.1016/J.JAAD.2014.04.001.

[52] E. Y. Lee, R. Alhusayen, P. Lansang, N. Shear, and J. Yeung, "What is hidradenitis suppurativa?," *Can. Fam. Physician*, vol. 63, no. 2, p. 114, Feb. 2017, Accessed: May 16, 2022. [Online]. Available: /pmc/articles/PMC5395382/.

[53] F. Farci and G. D. Mahabal, "Hyperkeratosis," *Encycl. Parasitol.*, pp. 1311–1311, Sep. 2021, doi: 10.1007/978-3-662-43978-4_1540.

[54] Kumar V, Abbas AK, Nelson Fausto, Robbins SL, and Cotran RS, "Robbins and Cotran's Pathological Basis of Disease," *Elsevier Heal. Sci.*, vol. 2, no. 9e, p. P. 1143, 2015, Accessed: May 17, 2022. [Online]. Available: https://www.semanticscholar.org/paper/Robbins-and-Cotran-pathologic-basis-of-disease-Kumar/d04446d9754f21f43ebfda30fb3d5702c29e97d2.

[55] R. M. Porter, J. Reichelt, D. P. Lunny, T. M. Magin, and E. B. Lane, "The Relationship Between Hyperproliferation and Epidermal Thickening in a Mouse Model for BCIE," *J. Invest. Dermatol.*, vol. 110, no. 6, pp. 951–957, Jun. 1998, doi: 10.1046/J.1523-1747.1998.00218.X.

[56] E. M. G. J. de Jong, P. E. J. van Erp, D. J. Ruiter, and P. C. M. van de Kerkhof, "Immunohistochemical detection of proliferation and differentiation in discoid lupus erythematosus," *J. Am. Acad. Dermatol.*, vol. 25, no. 6 Pt 1, pp. 1032–1038, 1991, doi: 10.1016/0190-9622(91)70303-J.

[57] P. Frost, G. D. Weinstein, and E. J. Van Scott, "The ichthyosiform dermatoses. II. Autoradiographic studies of epidermal proliferation," *J. Invest. Dermatol.*, vol. 47, no. 6, pp. 561–567, 1966, doi: 10.1038/JID.1966.185.

[58] F. W. Danby and L. J. Margesson, "Hidradenitis suppurativa," *Dermatol. Clin.*, vol. 28, no. 4, pp. 779–793, Oct. 2010, doi: 10.1016/J.DET.2010.07.003.

[59] D. Tang, R. Kang, C. B. Coyne, H. J. Zeh, and M. T. Lotze, "PAMPs and DAMPs: signal 0s that spur autophagy and immunity," *Immunol. Rev.*, vol. 249, no. 1, pp. 158–175, Sep. 2012, doi: 10.1111/J.1600-065X.2012.01146.X.

[60] K. V. Swanson, M. Deng, and J. P. Y. Ting, "The NLRP3 inflammasome: molecular activation and regulation to therapeutics," *Nat. Rev. Immunol.*, vol. 19, no. 8, pp. 477–489, Aug. 2019, doi: 10.1038/S41577-019-0165-0.

[61] N. Kelley, D. Jeltema, Y. Duan, and Y. He, "The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation," *Int. J. Mol. Sci.*, vol. 20, no. 13, Jul. 2019, doi: 10.3390/IJMS20133328.

[62] J. Korbecki *et al.*, "The Effect of Hypoxia on the Expression of CXC Chemokines and CXC Chemokine

Receptors-A Review of Literature," *Int. J. Mol. Sci.*, vol. 22, no. 2, pp. 1–30, Jan. 2021, doi: 10.3390/IJMS22020843.

[63] S. Mondal, N. Adhikari, S. Banerjee, S. A. Amin, and T. Jha, "Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview," *Eur. J. Med. Chem.*, vol. 194, p. 112260, May 2020, doi: 10.1016/J.EJMECH.2020.112260.

[64] C. Kapoor, S. Vaidya, V. Wadhwan, Hitesh, G. Kaur, and A. Pathak, "Seesaw of matrix metalloproteinases (MMPs)," *J. Cancer Res. Ther.*, vol. 12, no. 1, p. 28, Jan. 2016, doi: 10.4103/0973-1482.157337.

[65] A. Page-McCaw, A. J. Ewald, and Z. Werb, "Matrix metalloproteinases and the regulation of tissue remodelling," *Nat. Rev. Mol. Cell Biol.*, vol. 8, no. 3, pp. 221–233, Mar. 2007, doi: 10.1038/NRM2125.

[66] F. F. Albelwi *et al.*, "Halting Tumor Progression via Novel Non-Hydroxamate Triazole-Based Mannich Bases MMP-2/9 Inhibitors; Design, Microwave-Assisted Synthesis, and Biological Evaluation," *Int. J. Mol. Sci. 2021, Vol. 22, Page 10324*, vol. 22, no. 19, p. 10324, Sep. 2021, doi: 10.3390/IJMS221910324.

[67] G. A. Cabral-Pacheco *et al.*, "The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases," *Int. J. Mol. Sci. 2020, Vol. 21, Page 9739*, vol. 21, no. 24, p. 9739, Dec. 2020, doi: 10.3390/IJMS21249739.

[68] Alberts B, Johnson A, Lewis J, and et al., "Blood Vessels and Endothelial Cells - Molecular Biology of the Cell - NCBI Bookshelf," *National Library of Medicine, National Center for Biotechnology Information, New York Garland Science*, 2002. https://www.ncbi.nlm.nih.gov/books/NBK26848/ (accessed May 16, 2022).

[69] H. R. Mirzaei, "Adaptive Immunity," *Encycl. Infect. Immun.*, pp. 39–55, Jan. 2022, doi: 10.1016/B978-0-12-818731-9.00028-8.

[70] Janeway CA Jr, Travers P, Walport M, and et al., "The complement system and innate immunity - Immunobiology - NCBI Bookshelf," *National Library of Medicine, National Center for Biotechnology Information, New York Garland Science*, 2001. https://www.ncbi.nlm.nih.gov/books/NBK27100/ (accessed May 16, 2022).

[71] C. C. Zouboulis *et al.*, "Target molecules for future hidradenitis suppurativa treatment," *Exp. Dermatol.*, vol. 30 Suppl 1, no. S1, pp. 8–17, Jun. 2021, doi: 10.1111/EXD.14338.

[72] J. M. Fletcher, B. Moran, A. Petrasca, and C. M. Smith, "IL-17 in inflammatory skin diseases psoriasis and hidradenitis suppurativa," *Clin. Exp. Immunol.*, vol. 201, no. 2, pp. 121–134, Aug. 2020, doi: 10.1111/CEI.13449.

[73] K. Elkin, S. Daveluy, and K. Avanaki, "Review of imaging technologies used in Hidradenitis Suppurativa," *Ski. Res. Technol.*, vol. 26, no. 1, pp. 3–10, Jan. 2020, doi: 10.1111/SRT.12772.

[74] J. Sanchez *et al.*, "Matrix remodelling and MMP expression/activation are associated with hidradenitis suppurativa skin inflammation," *Exp. Dermatol.*, vol. 28, no. 5, pp. 593–600, May 2019, doi: 10.1111/EXD.13919.

[75] A. Tsaousi *et al.*, "MMP8 Is Increased in Lesions and Blood of Acne Inversa Patients: A Potential Link to Skin Destruction and Metabolic Alterations," *Mediators Inflamm.*, vol. 2016, 2016, doi: 10.1155/2016/4097574.

[76] J. Molnar *et al.*, "Hidradenitis Suppurativa and 1-Carbon Metabolism: Role of Gut Microbiome, Matrix

Metalloproteinases, and Hyperhomocysteinemia," *Front. Immunol.*, vol. 11, p. 1730, Aug. 2020, doi: 10.3389/FIMMU.2020.01730/BIBTEX.

[77] K. Henriksen and M. A. Karsdal, "Type I collagen," *Biochem. Collagens, Laminins Elastin Struct. Funct. Biomarkers*, pp. 1–12, Jan. 2019, doi: 10.1016/B978-0-12-817068-7.00001-X.

[78] M. Wieczorek and I. Walecka, "Hidradenitis suppurativa – known and unknown disease," *Reumatologia*, vol. 56, no. 6, p. 337, 2018, doi: 10.5114/REUM.2018.80709.

[79] S. Hessam, L. Scholl, M. Sand, L. Schmitz, S. Reitenbach, and F. G. Bechara, "A Novel Severity Assessment Scoring System for Hidradenitis Suppurativa," *JAMA Dermatology*, vol. 154, no. 3, p. 330, Mar. 2018, doi: 10.1001/JAMADERMATOL.2017.5890.

[80] C. C. Zouboulis *et al.*, "Development and validation of the International Hidradenitis Suppurativa Severity Score System (IHS4), a novel dynamic scoring system to assess HS severity," *Br. J. Dermatol.*, vol. 177, no. 5, pp. 1401–1409, Nov. 2017, doi: 10.1111/BJD.15748.

[81] E. A. Langan, A. Recke, T. Bokor-billmann, F. Billmann, B. K. Kahle, and D. Zillikens, "The Role of the Cutaneous Microbiome in Hidradenitis Suppurativa-Light at the End of the Microbiological Tunnel," *Int. J. Mol. Sci.*, vol. 21, no. 4, Feb. 2020, doi: 10.3390/IJMS21041205.

[82] T. Horiuchi, H. Mitoma, S. I. Harashima, H. Tsukamoto, and T. Shimoda, "Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents," *Rheumatology (Oxford).*, vol. 49, no. 7, pp. 1215–1228, Mar. 2010, doi: 10.1093/RHEUMATOLOGY/KEQ031.

[83] C. Fotiadou, E. Vakirlis, and D. Ioannides, "Spotlight on adalimumab in the treatment of active moderate-to-severe hidradenitis suppurativa," *Clin. Cosmet. Investig. Dermatol.*, vol. 9, p. 367, Oct. 2016, doi: 10.2147/CCID.S93619.

[84] M. M. Lowe *et al.*, "Immunopathogenesis of hidradenitis suppurativa and response to anti-TNF-α therapy," *JCI insight*, vol. 5, no. 19, Oct. 2020, doi: 10.1172/JCI.INSIGHT.139932.

[85] V. Jain and A. Jain, "Use of Lasers for the Management of Refractory Cases of Hidradenitis Suppurativa and Pilonidal Sinus," *J. Cutan. Aesthet. Surg.*, vol. 5, no. 3, p. 190, 2012, doi: 10.4103/0974-2077.101377.

[86] P. Lindsø Andersen *et al.*, "[Intense pulsed light treatment for hidradenitis suppurativa: a within-person randomized controlled trial]," *Eur. J. Dermatol.*, vol. 30, no. 6, pp. 723–729, Nov. 2020, doi: 10.1684/EJD.2020.3920.

[87] P. Babilas, S. Schreml, R. M. Szeimies, and M. Landthaler, "Intense pulsed light (IPL): a review," *Lasers Surg. Med.*, vol. 42, no. 2, pp. 93–104, Feb. 2010, doi: 10.1002/LSM.20877.

[88] A. Jfri *et al.*, "The Efficacy and Effectiveness of Non-ablative Light-Based Devices in Hidradenitis Suppurativa: A Systematic Review and Meta-Analysis," *Front. Med.*, vol. 7, p. 591580, Nov. 2020, doi: 10.3389/FMED.2020.591580/FULL.

[89] H. H. Van Der Zee, E. P. Prens, and J. Boer, "Deroofing: a tissue-saving surgical technique for the treatment of mild to moderate hidradenitis suppurativa lesions," *J. Am. Acad. Dermatol.*, vol. 63, no. 3, pp. 475–480, Sep. 2010, doi: 10.1016/J.JAAD.2009.12.018.

[90] Ø. Grimstad and G. Ingvarsson, "Carbon Dioxide Laser Treatment Using Methylene Blue-Assisted Sinus Tract Identification in Hidradenitis Suppurativa," *Dermatol. Surg.*, vol. 42, no. 11, pp. 1303–1304, Nov. 2016, doi: 10.1097/DSS.0000000000000824.

[91] P. R. Mikkelsen, D. N. Dufour, K. Zarchi, and G. B. E. Jemec, "Recurrence rate and patient satisfaction

of CO2 laser evaporation of lesions in patients with hidradenitis suppurativa: a retrospective study," *Dermatol. Surg.*, vol. 41, no. 2, pp. 255–260, Feb. 2015, doi: 10.1097/DSS.0000000000000264.

[92] A. Patel, "Anesthesia for Laser Airway Surgery," *Benumof Hagberg's Airw. Manag. Third Ed.*, pp. 824-858.e4, Jan. 2013, doi: 10.1016/B978-1-4377-2764-7.00040-3.

[93] Lumenis, "Long Pulsed Nd YAG Laser Technology | Lumenis," *Lumenis: Energy to healthcare*. https://lumenis.com/aesthetics/technology/long-pulsed-ndyag/ (accessed May 16, 2022).

[94] R. F. Magalhães *et al.*, "Consensus on the treatment of hidradenitis suppurativa - BrazilianSociety of Dermatology," *An. Bras. Dermatol.*, vol. 94, no. 2 Suppl 1, p. 7, Mar. 2019, doi: 10.1590/ABD1806-4841.20198607.

[95] N. Nosrati, L. Hunter, and E. Kelly, "Efficacy of Nd:YAG Laser for the Treatment of Hidradenitis Suppurativa," *J. Cosmet. Dermatological Sci. Appl.*, vol. 2012, no. 02, pp. 83–87, Jun. 2012, doi: 10.4236/JCDSA.2012.22019.

[96] M. Boury-Jamot *et al.*, "Expression and function of aquaporins in human skin: Is aquaporin-3 just a glycerol transporter?," *Biochim. Biophys. Acta*, vol. 1758, no. 8, pp. 1034–1042, Aug. 2006, doi: 10.1016/J.BBAMEM.2006.06.013.

[97] P. M. Tricarico *et al.*, "Aquaporins Are One of the Critical Factors in the Disruption of the Skin Barrier in Inflammatory Skin Diseases," *Int. J. Mol. Sci. 2022, Vol. 23, Page 4020*, vol. 23, no. 7, p. 4020, Apr. 2022, doi: 10.3390/IJMS23074020.

[98] J. M. Verbavatz *et al.*, "Skin Aquaporins: Function in Hydration, Wound Healing, and Skin Epidermis Homeostasis," *Handb. Exp. Pharmacol.*, vol. 190, pp. 205–217, 2009, doi: 10.1007/978-3-540-79885-9_10.

[99] A. F. Deyrieux and V. G. Wilson, "In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line," *Cytotechnology*, vol. 54, no. 2, pp. 77–83, Jun. 2007, doi: 10.1007/S10616-007-9076-1.

[100] I. Colombo *et al.*, "HaCaT Cells as a Reliable In Vitro Differentiation Model to Dissect the Inflammatory/Repair Response of Human Keratinocytes," *Mediators Inflamm.*, vol. 2017, 2017, doi: 10.1155/2017/7435621.

[101] K. Nakahigashi, K. Kabashima, A. Ikoma, A. S. Verkman, Y. Miyachi, and M. Hara-Chikuma, "Upregulation of aquaporin-3 is involved in keratinocyte proliferation and epidermal hyperplasia," *J. Invest. Dermatol.*, vol. 131, no. 4, pp. 865–873, 2011, doi: 10.1038/JID.2010.395.

[102] P. Kumar, A. Nagarajan, and P. D. Uchil, "Analysis of Cell Viability by the MTT Assay," *Cold Spring Harb. Protoc.*, vol. 2018, no. 6, pp. 469–471, Jun. 2018, doi: 10.1101/PDB.PROT095505.

[103] Merck, "MTT Assay Protocol for Cell Viability and Proliferation," *Merck KGaA*. https://www.sigmaaldrich.com/DK/en/technical-documents/protocol/cell-culture-and-cell-culture-analysis/cell-counting-and-health-analysis/cell-proliferation-kit-i-mtt (accessed May 18, 2022).

[104] T. L. Riss *et al.*, "Cell Viability Assays," *Assay Guid. Man.*, Jul. 2016, Accessed: May 16, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK144065/.

[105] T. Cai and M. E. Schmitt, "Characterization of Ribonuclease MRP Function," *Methods Enzymol.*, vol. 342, pp. 135–142, Jan. 2001, doi: 10.1016/S0076-6879(01)42541-9.

[106] M. Jalali, J. Zaborowska, and M. Jalali, "The Polymerase Chain Reaction: PCR, qPCR, and RT-PCR,"

Basic Sci. Methods Clin. Res., pp. 1–18, Jan. 2017, doi: 10.1016/B978-0-12-803077-6.00001-1.

[107] Thermo Fisher Scientific, "Traditional Methods of Cell Lysis," *Thermo Fisher Scientific*. https://www.thermofisher.com/dk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/traditional-methods-cell-lysis.html?adobe_mc=MCMID%7C61795603089405942172334046552374912651%7CMCAID%3D3137E277EAD0313B-40000ED5CBF0D0F9%7CMCORGID%3D5B135A0C5370E6B40A490D44%40AdobeOrg%7CTS=1614293705 (accessed May 17, 2022).

[108] D. C. Rio, M. Ares, G. J. Hannon, and T. W. Nilsen, "Purification of RNA using TRIzol (TRI reagent)," *Cold Spring Harb. Protoc.*, vol. 2010, no. 6, Jun. 2010, doi: 10.1101/PDB.PROT5439.

[109] Invitrogen, "SuperScript™ One-Step RT-PCR System with Platinum™ Taq DNA Polymerase," *Thermo Fisher Scientific*. May 10, 2016.

[110] D. Caetano-Anollés, "Polymerase Chain Reaction," *Brenner's Encycl. Genet. Second Ed.*, pp. 392–395, Jan. 2013, doi: 10.1016/B978-0-12-374984-0.01186-4.

[111] M. R. Green and J. Sambrook, "Analysis of DNA by Agarose Gel Electrophoresis," *Cold Spring Harb. Protoc.*, vol. 2019, no. 1, pp. 6–15, Jan. 2019, doi: 10.1101/PDB.TOP100388.

[112] M. Carter and J. C. Shieh, "Molecular Cloning and Recombinant DNA Technology," *Guid. to Res. Tech. Neurosci.*, pp. 207–227, Jan. 2010, doi: 10.1016/B978-0-12-374849-2.00009-4.

[113] R. Sarkar, S. Banerjee, S. A. Amin, N. Adhikari, and T. Jha, "Histone deacetylase 3 (HDAC3) inhibitors as anticancer agents: A review," *Eur. J. Med. Chem.*, vol. 192, p. 112171, Apr. 2020, doi: 10.1016/J.EJMECH.2020.112171.

[114] Y. J. Jiang, P. Kim, Y. F. Lu, and K. R. Feingold, "PPARgamma activators stimulate aquaporin 3 expression in keratinocytes/epidermis," *Exp. Dermatol.*, vol. 20, no. 7, pp. 595–599, Jul. 2011, doi: 10.1111/J.1600-0625.2011.01269.X.

[115] J. Xu, L. Yang, and L. Dong, "Tanshinol upregulates the expression of aquaporin 5 in lung tissue of rats with sepsis," *Oncol. Lett.*, vol. 16, no. 3, p. 3290, Sep. 2018, doi: 10.3892/OL.2018.9026.

[116] S. Luo *et al.*, "Tanshinol stimulates bone formation and attenuates dexamethasone-induced inhibition of osteogenesis in larval zebrafish," *J. Orthop. Transl.*, vol. 4, pp. 35–45, Jan. 2015, doi: 10.1016/J.JOT.2015.07.002.

[117] C. Gialeli, A. D. Theocharis, and N. K. Karamanos, "Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting," *FEBS J.*, vol. 278, no. 1, pp. 16–27, Jan. 2011, doi: 10.1111/J.1742-4658.2010.07919.X.

[118] A. Shah, R. Alhusayen, and S. Amini-Nik, "The critical role of macrophages in the pathogenesis of hidradenitis suppurativa," *Inflamm. Res.*, vol. 66, no. 11, pp. 931–945, Nov. 2017, doi: 10.1007/S00011-017-1074-Y.

[119] E. Mozeika, M. Pilmane, B. M. Nürnberg, and G. B. E. Jemec, "Tumour necrosis factor-alpha and matrix metalloproteinase-2 are expressed strongly in hidradenitis suppurativa," *Acta Derm. Venereol.*, vol. 93, no. 3, pp. 301–304, Mar. 2013, doi: 10.2340/00015555-1492/.

[120] N. L. Starodubtseva, V. V. Sobolev, A. G. Soboleva, A. A. Nikolaev, and S. A. Bruskin, "Genes expression of metalloproteinases (MMP-1, MMP-2, MMP-9, and MMP-12) associated with psoriasis,"

Russ. J. Genet. 2011 479, vol. 47, no. 9, pp. 1117–1123, Sep. 2011, doi: 10.1134/S102279541109016X.

[121] A. Michalak-Stoma, J. Bartosińska, D. Raczkiewicz, M. Kowal, D. Krasowska, and G. Chodorowska, "Assessment of Selected Matrix Metalloproteinases (MMPs) and Correlation with Cytokines in Psoriatic Patients," *Mediators Inflamm.*, vol. 2021, 2021, doi: 10.1155/2021/9913798.

[122] A. Mezentsev, A. Nikolaev, and S. Bruskin, "Matrix metalloproteinases and their role in psoriasis," *Gene*, vol. 540, no. 1, pp. 1–10, Apr. 2014, doi: 10.1016/J.GENE.2014.01.068.

[123] C. Xi, C. Xiong, H. Wang, Y. Liu, and S. Luo, "Combination of retinoids and narrow-band ultraviolet B inhibits matrix metalloproteinase 13 expression in HaCaT keratinocytes and a mouse model of psoriasis," *Sci. Reports 2021 111*, vol. 11, no. 1, pp. 1–7, Jun. 2021, doi: 10.1038/s41598-021-92599-w.

[124] G. B. Fields, "The Rebirth of Matrix Metalloproteinase Inhibitors: Moving Beyond the Dogma," *Cells 2019, Vol. 8, Page 984*, vol. 8, no. 9, p. 984, Aug. 2019, doi: 10.3390/CELLS8090984.

[125] T. Fischer and R. Riedl, "Inhibitory Antibodies Designed for Matrix Metalloproteinase Modulation," *Mol. 2019, Vol. 24, Page 2265*, vol. 24, no. 12, p. 2265, Jun. 2019, doi: 10.3390/MOLECULES24122265.

[126] N. Sela-Passwell *et al.*, "Antibodies targeting the catalytic zinc complex of activated matrix metalloproteinases show therapeutic potential," *Nat. Med.*, vol. 18, no. 1, pp. 143–147, Jan. 2012, doi: 10.1038/nm.2582.

[127] S. Löffek, O. Schilling, and C. W. Franzke, "Biological role of matrix metalloproteinases: a critical balance," *Eur. Respir. J.*, vol. 38, no. 1, pp. 191–208, Jul. 2011, doi: 10.1183/09031936.00146510.

[128] "MMP-8 (Matrix metalloproteinase-8)," *BioVendor R&D*. https://www.biovendor.com/mmp-8?utm_source=google&utm_medium=organic (accessed May 16, 2022).

[129] P. Garg, M. Vijay-Kumar, L. Wang, A. T. Gewirtz, D. Merlin, and S. V. Sitaraman, "Matrix metalloproteinase-9-mediated tissue injury overrides the protective effect of matrix metalloproteinase-2 during colitis," *Am. J. Physiol. - Gastrointest. Liver Physiol.*, vol. 296, no. 2, pp. 175–184, Feb. 2009, doi: 10.1152/AJPGI.90454.2008/ASSET/IMAGES/LARGE/ZH30110852380004.JPEG.

[130] H. Nagase, R. Visse, and G. Murphy, "Structure and function of matrix metalloproteinases and TIMPs," *Cardiovasc. Res.*, vol. 69, no. 3, pp. 562–573, Feb. 2006, doi: 10.1016/J.CARDIORES.2005.12.002/2/69-3-562-FIG3.GIF.

[131] J. E. Rundhaug, "Matrix Metalloproteinases, Angiogenesis, and Cancer," *Clinical Cancer Research | American Association for Cancer Research*, 2003. https://aacrjournals.org/clincancerres/article/9/2/551/203615/Matrix-Metalloproteinases-Angiogenesis-and (accessed May 19, 2022).

[132] C. Tallant, A. Marrero, and F. X. Gomis-Rüth, "Matrix metalloproteinases: Fold and function of their catalytic domains," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1803, no. 1, pp. 20–28, Jan. 2010, doi: 10.1016/J.BBAMCR.2009.04.003.

[133] K. E. Atkin *et al.*, "The Streptococcal Binding Site in the Gelatin-binding Domain of Fibronectin Is Consistent with a Non-linear Arrangement of Modules *," *J. Biol. Chem.*, vol. 285, no. 47, pp. 36977–36983, Nov. 2010, doi: 10.1074/JBC.M110.156935.

[134] M. Toth, A. Sohail, and R. Fridman, "Assessment of Gelatinases (MMP-2 and MMP-9) by Gelatin Zymography," *Methods Mol. Biol.*, vol. 878, pp. 121–135, 2012, doi: 10.1007/978-1-61779-854-2_8.

[135] K. Li, F. R. Tay, and C. K. Y. Yiu, "The past, present and future perspectives of matrix metalloproteinase

inhibitors," *Pharmacol. Ther.*, vol. 207, p. 107465, Mar. 2020, doi: 10.1016/J.PHARMTHERA.2019.107465.

[136] G. B. Fields, "Mechanisms of action of novel drugs targeting angiogenesis-promoting matrix metalloproteinases," *Front. Immunol.*, vol. 10, no. JUN, p. 1278, 2019, doi: 10.3389/FIMMU.2019.01278/BIBTEX.

[137] J. F. M. Pruijt *et al.*, "Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9)," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 96, no. 19, pp. 10863–10868, Sep. 1999, doi: 10.1073/PNAS.96.19.10863.

[138] G. Klein, E. Vellenga, M. W. Fraaije, W. A. Kamps, and E. S. J. M. De Bont, "The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia," *Crit. Rev. Oncol. Hematol.*, vol. 50, no. 2, pp. 87–100, May 2004, doi: 10.1016/J.CRITREVONC.2003.09.001.

[139] M. Raeeszadeh-Sarmazdeh, L. D. Do, and B. G. Hritz, "Metalloproteinases and Their Inhibitors: Potential for the Development of New Therapeutics," *Cells 2020, Vol. 9, Page 1313*, vol. 9, no. 5, p. 1313, May 2020, doi: 10.3390/CELLS9051313.

[140] Z. Puckrin, "Skin biopsy and ex vivo culture methods," *Reprocell*, 2022. https://www.reprocell.com/blog/biopta/skin-biospy-and-culture-methodology (accessed May 16, 2022).

[141] I. Hokstad, "Elektroforese," *Tidsskr. Den Nor. legeforening*, vol. 141, no. 3, Feb. 2021, doi: 10.4045/TIDSSKR.20.1041.

[142] T. Isbir, D. Kirac, B. Demircan, and B. Dalan, "Gel Electrophoresis," *Brenner's Encycl. Genet. Second Ed.*, pp. 165–167, Jan. 2013, doi: 10.1016/B978-0-12-374984-0.00580-5.

[143] X. C. Cheng, H. Fang, and W. F. Xu, "Advances in assays of matrix metalloproteinases (MMPs) and their inhibitors," *http://dx.doi.org/10.1080/14756360701511292*, vol. 23, no. 2, pp. 154–167, Apr. 2008, doi: 10.1080/14756360701511292.

[144] A. K. Leonard *et al.*, "Methods for the visualization and analysis of extracellular matrix protein structure and degradation," *Methods Cell Biol.*, vol. 143, pp. 79–95, Jan. 2018, doi: 10.1016/BS.MCB.2017.08.005.

[145] L. Kiczak *et al.*, "Expression and complex formation of MMP9, MMP2, NGAL, and TIMP1 in porcine myocardium but not in skeletal muscles in male pigs with tachycardia-induced systolic heart failure," *Biomed Res. Int.*, vol. 2013, 2013, doi: 10.1155/2013/283856.

[146] J. Christensen and V. P. Shastri, "Matrix-metalloproteinase-9 is cleaved and activated by Cathepsin K," *BMC Res. Notes*, vol. 8, no. 1, pp. 1–8, Jul. 2015, doi: 10.1186/S13104-015-1284-8/FIGURES/3.

[147] P. A. M. Snoek-van Beurden and J. W. Von Den Hoff, "Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors," *Biotechniques*, vol. 38, no. 1, pp. 73–83, 2005, doi: 10.2144/05381RV01/FORMAT/EPUB.

[148] E. Candrea, S. Senila, C. Tatomir, and R. Cosgarea, "Active and inactive forms of matrix metalloproteinases 2 and 9 in cutaneous melanoma," *Int. J. Dermatol.*, vol. 53, no. 5, pp. 575–580, 2014, doi: 10.1111/J.1365-4632.2012.05772.X.

[149] S. Inanc, D. Keles, and G. Oktay, "An improved collagen zymography approach for evaluating the collagenases MMP-1, MMP-8, and MMP-13," *Biotechniques*, vol. 63, no. 4, pp. 174–180, Oct. 2017, doi: 10.2144/000114597/.

[150] S. D. Gan and K. R. Patel, "Enzyme immunoassay and enzyme-linked immunosorbent assay," *J. Invest.*

Dermatol., vol. 133, no. 9, pp. 1–3, Sep. 2013, doi: 10.1038/JID.2013.287.

[151] W. Gaastra, "Enzyme-Linked Immunosorbant Assay (ELISA)," *SpringerLink, Proteins*, pp. 349–355, Nov. 1984, doi: 10.1385/0-89603-062-8:349.

[152] M. Alhajj and A. Farhana, "Enzyme Linked Immunosorbent Assay," *StatPearls*, Feb. 2022, Accessed: May 17, 2022. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK555922/.

[153] Thermo Fisher Scientific, "Overview of ELISA," *Thermo Fisher Scientific*. https://www.thermofisher.com/dk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html (accessed May 17, 2022).

[154] Thermo Fisher Scientific, "MMP8 Human ELISA Kit," *Invitrogen, Thermo Fisher Scientific*. https://www.thermofisher.com/elisa/product/MMP8-Human-ELISA-Kit/EHMMP8?ef_id=CjwKCAjw682TBhATEiwA9crl35tVKEis7Jr2mdlebE58fdRAFbcQSC-Hc2Mc4MJ3LODFistSCfJMdhoCnOEQAvD_BwE:G:s&s_kwcid=AL!3652!3!313682207674!b!!g!!%2Bhuman%2Bmmp%2B8%2Belisa&cid=bid_pca_iel_r01_co_cp1359_pjt0000_bid00000_0se_gaw_nt_pur_con&gclid=CjwKCAjw682TBhATEiwA9crl35tVKEis7Jr2mdlebE58fdRAFbcQSC-Hc2Mc4MJ3LODFistSCfJMdhoCnOEQAvD_BwE (accessed May 17, 2022).

[155] Thermo Fisher Scientific, "MMP2 Human ELISA Kit - Invitrogen," *Invitrogen, Thermo Fisher Scientific*. https://www.thermofisher.com/elisa/product/MMP2-Human-ELISA-Kit/KHC3081 (accessed May 21, 2022).

[156] Thermo Fisher Scientific, "MMP9 Human ELISA Kit - Invitrogen," *Invitrogen, Thermo Fisher Scientific*. https://www.thermofisher.com/elisa/product/MMP9-Human-ELISA-Kit/BMS2016-2 (accessed May 21, 2022).

[157] "Antibiotic resistance," *World Health Organization*, Jul. 31, 2020. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (accessed May 22, 2022).

[158] M. Grzesiak, K. Kaminska, K. Knapczyk-Stwora, and A. Hrabia, "The expression and localization of selected matrix metalloproteinases (MMP-2, -7 and -9) and their tissue inhibitors (TIMP-2 and -3) in follicular cysts of sows," *Theriogenology*, vol. 185, pp. 109–120, Jun. 2022, doi: 10.1016/J.THERIOGENOLOGY.2022.03.029.

[159] C. J. Smith and A. M. Osborn, "Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology," *FEMS Microbiol. Ecol.*, vol. 67, no. 1, pp. 6–20, Jan. 2009, doi: 10.1111/J.1574-6941.2008.00629.X.

[160] Thermo Fisher Scientific, "Endpoint PCR, quantitative PCR and digital PCR - Scientific Videos | Thermo Fisher Scientific US," *Thermo Fisher Scientific*. https://videos.thermofisher.com/detail/video/4896017367001/endpoint-pcr-quantitative-pcr-and-digital-pcr (accessed May 22, 2022).

[161] Z. Ren, J. Chen, and R. A. Khalil, "Zymography as a Research Tool in the Study of Matrix Metalloproteinase Inhibitors," *Methods Mol. Biol.*, vol. 1626, p. 79, 2017, doi: 10.1007/978-1-4939-7111-4_8.

[162] G. Chaudhari, "(PDF) AN OVERVIEW ON ELISAS TECHNIQUES FOR ANALYTE DETECTION

TO NEW LEVELS: A REVIEW," *INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH*, *Researchgate*, 2014. https://www.researchgate.net/publication/281100220_AN_OVERVIEW_ON_ELISAS_TECHNIQUES_FOR_ANALYTE_DETECTION_TO_NEW_LEVELS_A_REVIEW (accessed May 16, 2022).

Appendix

Appendix 1:

Components of the Innate Immune system, created in BioRender.com.

Components of the Innate Immune System

	Cell Type	Function
	Neutrophil	Phagocytose and destroy microbes from outside
	Eosinophil	Release toxic proteins and free radicals, which kill bacteria and parasites
	Basophil	Release chemicals to stimulate inflammatory responses
	Macrophage	Phagocytic cells capable of ingesting and destroying microbes and antigen presentation to T cells
	Dendritic cell	Activate the cells of the adaptive immune system and destroy invading microbes through phagocytosis
	Mast cell	Produce inflammatory molecules
	Natural killer cell	Identify and destroy infected cells