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A microRNA focus on acne

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Abstract

Acne (syn. acne vulgaris) is a common inflammatory skin disorder associated with puberty and adolescence. Driven by complex interactions between the pilosebaceous unit and *Cutibacterium acnes* (*C. acnes*) bacteria, the disease is characterised by comedonal lesions, papules, pustules and nodules that appear predominantly on the face. Acne and sequelae such as scarring and pigment changes affect health-related quality of life negatively. Approvals for nucleic acid therapies (NATs) such as short-interfering RNA (siRNA) drugs and antisense oligonucleotides (ASOs) have surged in recent years, for rare disorders with little or no effective treatments. These advances, along with clinical trials for microRNA (miRNA) modulation in skin contexts, raise the possibility that NATs may have potential for future acne treatment regimens. In this review, we highlight potential miRNA targets for anti-acne therapy. We provide a brief overview of acne pathophysiology and highlight roles of *C. acnes.* We then focus on recently discovered differential effects of planktonic and biofilm *C. acnes* on a Toll-like receptor 2 (TLR2) axis spanning miR-146a-5p. We appraise miR-146a-5p in sebocytes before addressing the putative contributions of miR-21-5p, miR-233-3p and miR-150-5p to inflammatory axes in acne. We conclude with translational perspectives and considerations of patient involvement in miRNA-related research for acne.

Introduction

Human skin provides a protective barrier for the body, shielding against chemical, mechanical and microbial assaults (1). *Cutibacterium acnes (C.acnes* - formerly *Proprionibacterium acnes*)*,* is a dominant commensal bacterium that forms part of the normal microbiota of healthy skin (2-4) but also drives inflammatory processes that give rise to characteristic clinical lesions in acne i.e. papules, pustules and nodules (5). The Global Burden of Skin Disease study found that acne vulgaris was the 8th most prevalent disease worldwide in 2010, with the vast majority of acne-related years lost due to disability (YLD) occurring between the ages of 10 and 40 (6). The prevalence in adolescents ranges from the ~35% reported recently for Egypt (7), to around 50% in Serbia (8) and Montenegro (9), around 60% in the UK (10), Turkey (11, 12) and Japan (13) and over ~90% in Brazil (14), Belgium, China (Hong Kong) and Iran; see (15) and references therein. An analysis of acne incidence rates in teenagers aged 15-19 years revealed an upward trajectory in all regions examined except Sub-Saharan Africa (16). Further, the comparative prevalence of acne in 15–19-year-olds was up to ~1.5 fold higher in developed countries compared to developing ones, though whether this difference is statistically significant was not entirely clear (16).

Acne lesions most commonly occur on the face, where they are arguably of greatest concern, but in over 50% of those affected, lesions also appear on the trunk (17). The resulting burden of skin

disease may vary by patient demographics, access to treatments and duration of the disease. Acne symptoms and sequelae contribute physically and psychosocially to the burden of disease, as do the costs of management. Acne typically presents in adolescence at a time of significant transition, with a strong impact on interpersonal relationships, social functioning and mental health (18). The high prevalence of acne also presents an economic burden for society and widespread, prolonged use of antibiotics introduces a potential added burden through resulting antimicrobial resistance (5).

Recently, the long-heralded promise of RNA interference for silencing gene expression in disease has been realised, with the approval of antisense oligonucleotide (ASO) and short-interfering RNA (siRNA) for various rare metabolic diseases. The former include nusinersen (Spinraza®) for spinal muscular atrophy (19, 20), casimersen for Duchenne muscular dystrophy (21, 22) and volanesorsen for familial chylomicronemia syndrome (23). Approved siRNA drugs include patisiran (24), givosiran (Givlaari®) (25), lumasiran (Oxlumo®) (26), inclisiran (Leqvio®) (27) and Amvuttra® (vutrisiran) (28). The success of these therapies heralds a new dawn for the use of nucleic acid therapies (NATs) to improve clinical outcomes in patients (29).

Considering the skin is accessible to topical active agents, NATs may conceivably gain future application in common skin disorders such as acne. One area of interest in this regard relates to microRNAs (miRNAs), small non-coding RNAs ~22 nucleotides long that are structurally and functionally similar to siRNAs. While the potential of miRNA replacement or miRNA inhibition has gained traction for skin disorders such as chronic wounds (30-33) and hypertrophic scarring (34), miRNA-directed approaches for acne and other inflammatory dermatoses that predominate the face of adolescents and young adults has received limited attention. Here we review a selection of such miRNAs associated with acne. We focus on miR-146a given recent studies linking this miRNA to *C. acne* biofilms (35) and miR-21, miR-150 and miR-233, all of which were elevated in acne lesions compared to normal skin according to a recent report from Layton and colleagues (36). We propose the exploitation of recent developments in oligonucleotide delivery to the skin as a tractable approach for developing miRNA-directed topical agents for acne therapy.

The pathophysiology of acne

The pathophysiology of acne, which has been summarised elsewhere (16, 37, 38) centres on the effects of *C. acnes* on the pilosebaceous unit (PSU), as depicted in Figure 1. The name "pilosebaceous" derives from the Latin *plius* for hair, and *sebum* for grease (39). The PSU consists of a hair follicle and sebaceous gland as the minimal unit, along with the arrector pili muscle. The sebaceous gland provides a lipid-rich environment in which *C. acnes* can thrive given it is an anaerobic bacterium that can degrade triglycerides in sebum to release free fatty acids that support

bacterial adherence, as reviewed (40). Sebum production can be triggered through pathways associated with the dihydrotestosterone (DHT) receptor, histamine receptor, neuromodulator receptor, leptin receptor, insulin-like growth factor receptor and peroxisome proliferator-activated receptors (37). The DHT agonists testosterone, dihydrotestosterone and dehydroepiandrosterone sulphate are particularly important drivers of sebum production during puberty. No correlation between androgen levels and acne severity has been established, however, and the constituents of sebum, not just the quantity, appear to play a role in acne pathogenesis (41). Androgens boost proliferation of hair follicle keratinocytes and sebocytes within the PSU, which manifests as increased facial grease production in early puberty (42). These androgens also enhance proliferation of ductal lining cells in the hair follicle lumen and accumulate to form a plug through mechanisms thought to involve *C. acnes* biofilms (43). In the meantime, sebum production and hair growth continue within the PSU, the anoxic conditions and abundance of fatty acids creating ideal conditions for *C. acnes* to flourish. Eventually, the plugged PSU bursts, stimulating an immunological response that ultimately generates an erythematous pustule (16).

Biofilm formation by *C. acnes*

The biofilm-forming ability of *C. acnes* and its impact on the pathogenesis of human disease has been reviewed recently by Coenye and colleagues (44). While considerable evidence is available for *C. acnes* biofilm growth and establishment of infection on abiotic surfaces such as prosthetics and implants, the occurrence and role of *C. acnes* biofilms in acne is less well established. However, this is not necessarily indicative of a correlation between *C. acnes* biofilm formation and anatomical site – while a slightly greater biomass and biofilm thickness has been noted to result from *C. acnes* isolates obtained from deep tissue/implant infections compared to those sourced from skin, biofilm formation in fact appears to correlate more closely with *C. acnes* phylotype rather than site of isolation (45).

Early studies demonstrated the ability of *C. acnes* isolates to grow as biofilms *in vitro* (46), and proposed that the sticky biofilm matrix components secreted into sebum may drive adhesion and accumulation of keratinocytes within the hair follicle and subsequent acne plug formation (43). *In vitro*-cultured *C. acnes* biofilms were also noted to produce larger amounts of virulence factors such as lipases and the quorum sensing molecule autoinducer-2 relative to planktonic cells, and demonstrated greater resistance to anti-infective agents than cells in suspension (46).

In support of *in vitro* observations, *C. acnes* biofilms have been identified in biopsy samples from facial skin of acne patients and noted to occur more frequently in the PSU of individuals with acne compared to acne-free controls (47). Biofilm morphology varied even within the same hair follicle, with patterns of follicular attachment, adherence to the hair shaft, full-spreading and

centralised sections of biofilm all noted. Biofilms were also deemed to have the potential to extend over distances as large as a millimetre, presenting considerable challenges for treatment (48). While a paucity of PSU-relevant *in vivo* models has hindered in-depth study of acne-specific *C. acnes* biofilms, a hair follicle-like environment has recently been successfully demonstrated in the gut of germ-free, lipid-fed fruit flies (49). The ready growth of *C. acnes* biofilm in this model renders it a promising tool for studying the impact of therapeutic interventions on acne-associated *C. acnes* biofilm.

A toll-like receptor 2: MicroRNA-146a-5p axis in acne keratinocytes

Toll-like receptors (TLRs) serve as first responders to microbial assault, recognising a plethora of pathogen-associated molecular patterns (PAMPs) associated with bacteria, fungi and viruses (50). Early work showed PAMPs including TLR2 agonist Pam3CSK4, TLR4 agonist lipopolysaccharide (LPS) and TLR5 agonist flagellin induce miR-146 in THP-1 monocytes, as did proinflammatory cytokines IL-1β and TNFα (51). The induction of miR-146 was dependent on NF-κB, a major transcription factor downstream of TLR, IL-1 and TNF receptors. In turn, miR-146 targeted IRAK1 and TRAF6, two crucial signalling enzymes for TLR/IL-1 signalling to NF-κB (52). This feedback loop from TLR2 signalling to miR-146a-dependent repression of signalling mediators is illustrated in Figure 2, along with other miRNA:target interactions with translational potential. Importantly, subsequent work from the Pivarcsi group showed TLR2 agonists evoked sustained miR-146a-5p expression in human epidermal keratinocytes and suppressed expression of IRAK1, TRAF6 and downstream proinflammatory molecules IL-8, CCL20 and TNFα (53).

Both heat-killed *C. acne* biofilms and their planktonic counterparts enhanced TLR2 expression (2–3 fold) in keratinocytes (35). In contrast, *C. acne* biofilms, but not the planktonic forms, raised the expression of inflammatory cytokines IL-6, IL-8 and TNFα, and this was observed at both the mRNA and protein levels. Hence while both forms of *C. acnes* contain PAMPs that stimulate TLR2 signalling pathways, it seems there are alternative/additional mechanisms that specifically impinge on the TLR2 but not the IL-6, IL-8 and TNFα promoters.

Importantly, exposure to heat-killed *C. acne* biofilms triggered miR-146a-5p expression in keratinocytes, with a 4-fold increase in miR-146a after 24 hours (35). Induction of miR-146a-5p was dependent on TLR2, MyD88, NF-κB and ERK as pharmacologic inhibition of these proteins abrogated miR-146a-5p induction. Interestingly, miR-146a-5p was distributed throughout the interfollicular epidermis, perifollicular epidermis and pilosebaceous ducts of lesions. As expected, given miR-146a-5p inhibits expression of IRAK and TRAF6, treatment of keratinocytes with a miR-

146a-5p mimic reduced *C.acnes*-biofilm dependent induction of IL-6, IL-18 and TNFα (35). Thus miR-146a-5p enhancement may be useful for dampening inflammation in acne (Fig. 1).

MicroRNA-146a-5p axis in sebocytes

While the above studies focussed on keratinocytes, very recent work by Törőcsik and colleagues now shows that both TLR2 and TLR4 agonists induced miR-146a-5p by ~2.5 fold in SZ95 human sebocytes after 24 h treatment (54). Importantly, miR-146a-5p appeared to be elevated in the sebaceous glands of acne skin samples and a miR-146a-5p mimic reduced IL-8 secretion by over 50% in sebocytes. Consistent with this, the ability of culture media from miR-46a-loaded sebocytes to promote chemoattraction of peripheral blood mononuclear cells was almost abolished (54). Hence a miR-146a-5p mimic may have translational potential to support resolution of acne-related inflammation. Interestingly, Törőcsik and co-workers identified GNG7 (G protein subunit gamma 7) as one of the most highly elevated genes following miR-146a-5p inhibition (54). However, GNG7 does not appear to be a direct target of miR-146a (based on a search of TargetScan 7.1). In addition, although the authors propose a model in which miR-146a indirectly affects the lipid content of sebocytes via GNG7, a miR-146a mimic had no effect on sebocyte lipid content suggesting that raised miR-146a has a net neutral effect on sebaceous lipid homeostasis.

MicroRNA-21-5p: an established player in keratinocyte inflammation

Perhaps the strongest evidence implicating miR-21-5p in skin inflammation comes from the Wagner study linking miR-21-5p-dependent repression of TIMP3 (tissue inhibitor of metalloproteinase 3) in epidermal keratinocytes to enhanced release of soluble, active TNFα from its membrane-bound precursor (55). However, the cellular distribution of miRNAs in the acne lesions have not been established, so we do not know which cell types are primarily responsible for elevated levels of miR-21-5p and the other miRNAs (miR-150-5p, miR-223-3p) that appeared to be elevated in acne lesions (36). Nonetheless, inhibition of miR-21-5p in acne lesions may be a tractable method for lowering active TNFα levels. This may in turn ameliorate disease given case reports of severe acne being treated effectively with the anti-TNF biologics etanercept and adalimumab (56, 57).

MicroRNA-150-5p: a potential immunological role in acne?

The roles of miR-150-5p in the skin have received little attention but what is well-established is that miR-150-5p is dynamically regulated during lymphoid and myeloid cell differentiation (58). The elevated expression of miR-150-5p in acne lesions reported by Layton and colleagues (36) may thus reflect the infiltration of T helper cells. Interestingly, Kim and colleagues defined a protective subset

of IL-10^{+}IL-17^{+}IL-22^{+}IL-26^{+} Th17 cells with robust bactericidal activity induced by *P. acne* strains associated with healthy skin (59). Notably, there is evidence that miR-150-5p can modulate IL-10 expression, the direction of the effect depending on cellular context (60, 61). Thus, the relationship between miR-150-5p and IL-10 expression in Th17 cells needs to be established in order to evaluate the potential of miR-150 as a novel target for acne therapy.

Is miR-223-3p a relevant target for acne?

Early studies showed miR-223 regulates the proliferation and differentiation of granulocyte precursors in a mouse model (62). More recent work suggests that miR-223-3p binds directly to the 3′UTR of IL-6 transcripts to dampen IL-6 secretion from neutrophils during wound repair (63). Further, at least by luciferase reporter assay, miR-223-3p was shown to directly repress several key mediators of TLR-dependent NF-κB activation, including *Cul1a, Cul1b, Traf6, and Tab1* (64). Loss of miR-223 raised *Cul1a* and *Traf6* transcript levels modestly but significantly in wounded zebrafish embryos (64). Given the inflammatory status of acne lesions, it is tempting to speculate that elevated miR-223 in acne lesions may be a compensatory mechanism aimed at restoring homeostasis. In that regard, it will be important to determine whether a synthetic miR-223 mimic can abrogate the reported DHT-dependent induction of IL-6 in cultured sebocytes (65) as well as *C.-acnes*-dependent activation on NF-κB.

Taming toll-like receptor 2

As an alternative to targeting downstream signalling mediators, can miRNA be used to lower the expression of TLR2 itself? Early work in fibroblast-like synoviocytes (FLS) from rheumatoid arthritis (RA) patients had shown that miR-19a and miR-19b mimics directly repress TLR2 (66). As a result, upregulation of IL-6 mRNA by TLR2 agonists was almost completely abolished in FLS loaded with miR-19a or miR-19b mimics (66). More recent independent studies confirmed these observations in FLS (67). On the other hand, early investigations found miR-19b mimics enhance activation of RA FLS by targeting negative regulators of NF-κB activation (68). In any case, the impact of miR-19 mimics on TLR2-mediated signalling in acne remains to be established.

From Discovery to miRNA-directed therapy

Although liquid phase oligonucleotide synthesis (LPOS) was demonstrated early on (69-71), solid-phase oligonucleotide synthesis (SPOS) based on phosphoramidite chemistry has been the main production method for GMP-grade oligonucleotides. However, due to scalability limitations, high reagent consumption and the large quantities that are anticipated as NATs move beyond rare diseases

into conditions with relatively high prevalence, the race is on to develop LPOS that can meet demand in a cost-effective fashion (72). As such, we may soon witness NATs reach affordability levels that will see them deployed in common, non-life-threatening disorders like acne. The translational challenges for miRNA-based therapeutics should not be underestimated, however. For instance, the first miRNA replacement therapy to reach clinical trials, systemic administration of a liposomal formulation of miR-34a mimic, was terminated early due to serious adverse immune-related reactions in patients with advanced solid tumours, though no such immunological responses were predicted in toxicological studies that included non-human primates (73). On the other hand, clinical trials with a miR-29b mimic (remlarsen) delivered via intradermal injection found remlarsen was safe and well tolerated, with no severe adverse events (34).

One additional factor to consider is the cost of NATs. Current approved ASOs and siRNAs range from 300k–750k US dollars per year (29). However, these drugs were generally developed for orphan and rare diseases and often involved what were novel chemistries at the time. In contrast, the mRNA vaccines against COVID19 cost 30–40 US dollars per treatment. Given the high prevalence of acne, cost-effective miRNA-directed interventions may be economically feasible.

Targeting microRNA-directed therapies to the pilosebaceous unit

Multiple agents have been explored for targeting miRNA to the epidermis, including cell penetrating peptides, spherical nucleic acids, deformable liposomes and liquid crystalline nanodispersions, as reviewed elsewhere (74). More recently, ionic liquids have also been evaluated for oligonucleotide delivery to the skin, with Mitragotri and colleagues showing safe and effective delivery of siRNA (75). However, the utility of the aforementioned delivery vehicles for topical delivery of miRNA mimics such as miR-146a mimic for acne treatment remains to be established. Application of such miRNA mimics directly to the affected areas could be used in combination with antibiotics or oral isotretinoin to accelerate the clearance of acne lesions.

Interestingly, siRNA, miRNA mimics and miRNA inhibitors have long been conjugated to cholesterol to improve uptake. Recently, alternative lipids such as eicosapentaenoic acid (EPA) and docosanoic acid (DCA), have also been explored (76). Given the lipophilic nature of the sebaceous gland, could the use of such lipid:miRNA conjugates represent a tractable organ for the development of next-generation acne treatments that directly reduce inflammation via miRNA-directed mechanisms? However, more work is needed in relation to the cell-specific localisation of miRNAs in acne lesions.

It is also worth noting that there are small molecule inhibitors of TLR signalling proteins IRAK4, IRAK1, TRAF6 and TAK1 that could also be deployed for inhibition of biofilm-derived *C.*

acnes inflammation. Conversely, there may be a case for combinatorial approaches, where miRNA mimics are used to decrease expression of IRAK1 and TRAF6 while the pharmacologic inhibitors reduce residual activity of the inflammatory pathway.

Engaging patients in acne research

Given the chronic nature of acne and prolonged treatments, the question that arises is whether miRNA-directed agents could be used as an alternative to some current therapies where not tolerated or indicated. It will be important to determine whether such miRNA-targeted interventions could shorten the duration of acne and potentially reduce resultant scarring and pigment changes. Clearly, there is a need for robust engagement of acne patients with emerging trends in miRNAs and NATs to capture the patient voice for putative development of such miRNA therapeutics for acne. This is pertinent because although antibiotics remain a mainstay for acne treatment, prescribing habits demonstrate overuse and there are concerns about this due to the emergence of antimicrobial resistance (77). Many acne patients receive prolonged courses of antibiotics and current guidelines now recommend judicious and limited use (78). There is evidence that acne scarring correlates with duration of disease and therefore implementing early effective therapy is required to mitigate sequelae (79, 80). Oral isotretinoin is a highly effective therapy licensed for severe acne including acne at the risk of permanent scarring but there are significant concerns about possible and potential adverse effects which can limit prescribing and it is not licensed for mild to moderate disease (78).

Notably, in terms of patient involvement in the co-production of knowledge, a James Lind Alliance Priority Setting Partnership (PSP) for acne recently made a number of recommendations (81). These included "*What management strategy should be adopted for the treatment of acne in order to optimise short and long-term outcomes*?" "*What is the best treatment for acne scars?"* and "*What is the correct way to use antibiotics in acne to achieve the best outcomes with least risk?"* In this regard, a reduction in topical and oral antibiotic prescribing for acne has been proposed, notably as part of a broader strategy for constraining antibiotic resistance in acne (82, 83). We suggest that with future exploration, miRNA-targeted interventions could provide an alternative topical approach to acne management and address some of the challenges which were identified by relevant personnel in the priority setting partnership. Patients will need to be appropriately engaged with the RNA therapeutics knowledge domain if this area of research is to progress and undergo clinical trials.

Conclusions

With the therapeutic potential of NATs for rare disorders now firmly established, consideration can be given to RNA-directed therapeutics for non-life-threatening conditions like acne. Safety will be a

crucial consideration given the adverse effects associated with approved siRNA drugs (84). Any miRNAs or miRNA inhibitors for acne will be administered topically rather than via the subcutaneous or intravenous routes used for currently approved siRNA drugs. Hence, any putative adverse effects may be confined to the skin, though systemic risks cannot be completely excluded. In any case, effective delivery of stabilised miRNA mimics across the epidermal barrier into keratinocyte and sebocyte cytosols needs to be rigorously established to enable miRNA-dependent amelioration of acne. Such approaches may accelerate the resolution of the disease at molecular, cellular and aesthetic levels, improving both cutaneous and psychological health and well-being.

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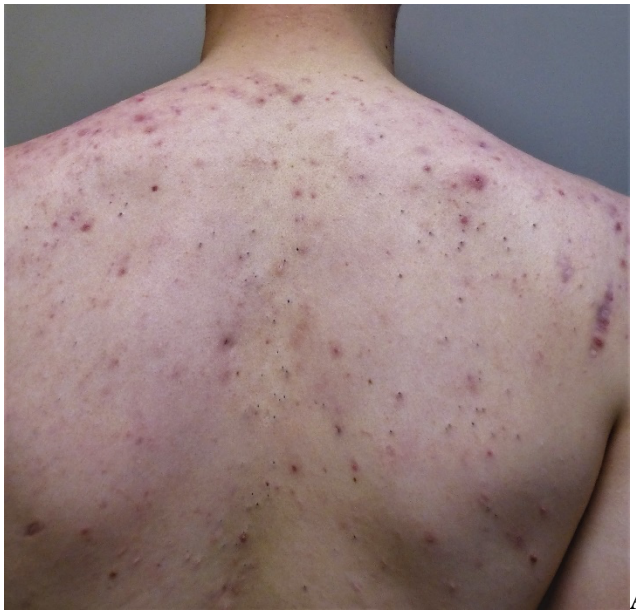
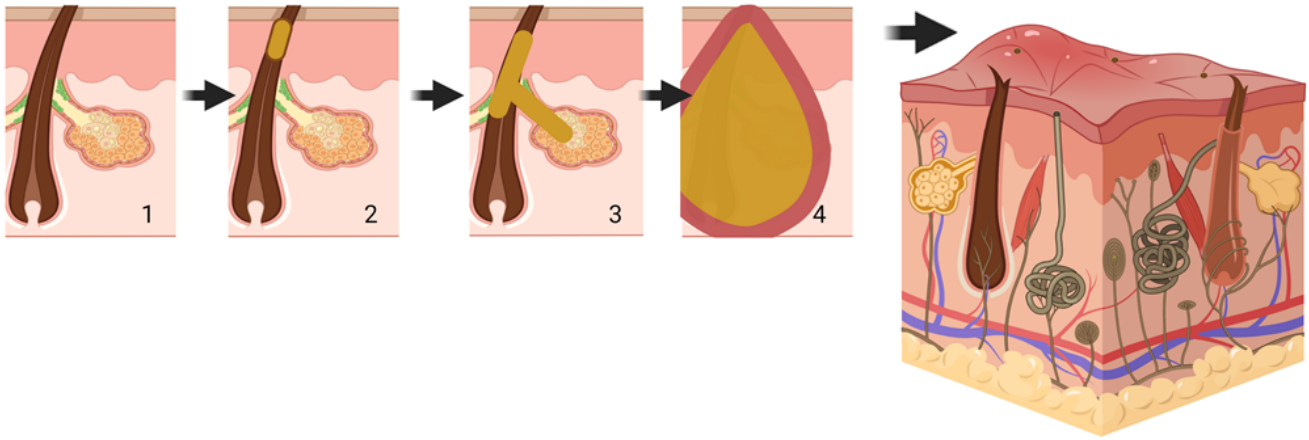


A)



B)

Figure 1. The pathophysiology of acne. A) A case of severe acne on the back; B) In the healthy pilosebaceous unit (PSU), egress of sebum from the hair follicle occurs without restriction (1). Formation of a plug through complex interactions between hair follicle cells, sebum and *C. acnes* blocks the pore (2). This in turn supports *C. acnes* proliferation and drives sebum and bacterial accumulation in the follicular canal (3). The surfeit of material in the PSU eventually causes the rupture of the follicular canal, triggering the inflammation that presents clinically as acne swellings. Created in BioRender.

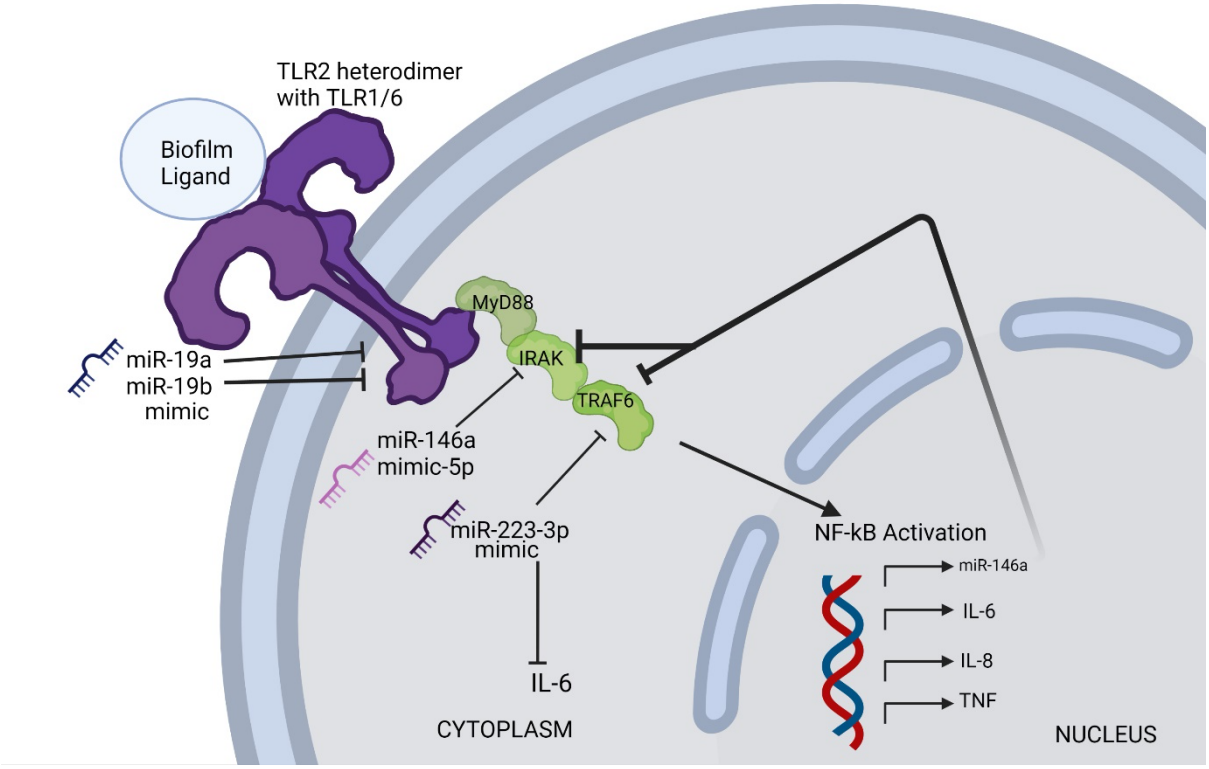


Figure 2. Reducing acne-related inflammation by miRNA elevation. *C. acnes* biofilms trigger TLR2 signalling that leads to the induction of miR-146a and inflammatory cytokines downstream of NF-κB and other transcription factors (not shown). Upregulation of miR-146a serves as an endogenous feedback mechanism to dampen inflammation. Candidate targets for the development of miRNA mimic therapeutics that may silence inflammatory signalling are shown. IL, interleukin; NF-κB, nuclear factor kappa B; TLR, Toll-like receptor; TNF, tumour necrosis factor alpha. Created in BioRender.