Review

Acne Transcriptomics: Fundamentals in Acne Pathogenesis and Isotretinoin Treatment

Bodo C. Melnik *

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Review

Acne Transcriptomics: Fundamentals in Acne Pathogenesis and Isotretinoin Treatment

Bodo C. Melnik

Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, Osnabrück, Germany; melnik@t-online.de

* Correspondence: melnik@t-online.de; Tel.: +495241988060

Abstract: This review provides information on acne transcriptomics allowing deeper insights into acne pathogenesis and isotretinoin´s mode of action. Puberty-induced insulin-like growth factor 1 (IGF-1), insulin and androgen signaling activate the kinase AKT and mechanistic target of rapamycin complex 1 (mTORC1). Western diet (hyperglycemic carbohydrates, milk/dairy products) as well co-stimulate AKT/mTORC1 signaling. AKT-mediated phosphorylation of nuclear FoxO1 and FoxO3 results in their extrusion into the cytoplasm, a critical switch, which enhances the transactivation of lipogenic and proinflammatory transcription factors including androgen receptor (AR), sterol regulatory element-binding transcription factor 1 (SREBF1), peroxisome proliferator-activated receptor γ (PPARγ), and signal transducer and activator of transcription 3 (STAT3) but reduces FoxO1-dependent expression of GATA binding protein 6 (GATA6), the key transcription factor of infundibular keratinocyte homeostasis. AKT-mediated phosphorylation of the p53-binding protein MDM2 promotes the degradation of p53. In contrast, isotretinoin enhances the expression of p53, FoxO1 and FoxO3 in sebaceous glands of acne patients. Overexpression of these proapoptotic transcription factors explains isotretinoin´s desired sebum-suppressive effect via induction of sebocyte apoptosis but also its adverse effects including teratogenicity (neural crest cell apoptosis), reduced ovarian reserve (granulosa cell apoptosis), risk of depression (apoptosis of hypothalamic neurons), VLDL hyperlipidemia, intracranial hypertension and dry skin.

Keywords: acne; apoptosis; FoxO1; FoxO3; isotretinoin; mTORC1; p53; pathogenesis; therapy; transcriptomics

1. Introduction

In recent years, tremendous scientific progress has been made in unraveling the transcriptional regulation in acne vulgaris. This review provides updated information on acne transcriptomics allowing deeper insights into the pathogenesis and treatment of acne. Acne vulgaris is a very common chronic inflammatory skin disorder with a complex pathogenesis. Four factors play vital roles in acne pathophysiology: hyperseborrhea and dysseborrhea, altered keratinization of the pilosebaceous duct (comedogenesis), effects mediated by *Cutibacterium acnes* (*C. acnes*) and Th17 cell-driven inflammation [1,2].

Isotretinoin is the most efficient anti-acne drug improving all major factors of acne pathogenesis. This review focuses on three key transcription factors, p53, FoxO1 and FoxO3, whose suppression plays important roles in acne pathogenesis, whereas their upregulation by isotretinoin explains isotretinoin´s beneficial as well as its adverse effects in the treatment of acne.

2. Growth Factor Signaling in Acne Activating the Kinase AKT

Various growth factors induce acne pathogenesis. Their input signals converge in the activation of the kinase AKT (protein kinase B), which modifies the activity and expression of important transcription factors promoting the disease.

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2.1. Insulin-Like Growth Factor 1

Insulin-like growth factor 1 (IGF-1) is a potent mitogen [3]. IGFs bind specifically to the IGF-1 receptor (IGF1R) on the cell surface of targeted tissues [4]. During puberty increasing production of growth hormone (GH) leads to the activation of the GH/IGF-1 axis [5]. IGF-1 is the key hormone of puberty [6] and promotes sexual differentiation and linear bone growth [7]. Acne becomes manifest at a relatively late stage of puberty, at or shortly after peak height velocity in boys and menarche in girls [8]. Deplewski and Rosenfield [9] emphasized the important role of IGF-1 in the development of the pilosebaceous unit. Increased serum IGF-1 levels correlate with acne lesion counts in females [10] and increased sebum secretion rates in male acne patients [11]. IGF-1 is overexpressed in epidermis and pilosebaceous units of acne patients compared to controls [12]. Western diet, especially hyperglycemic carbohydrates [13], milk and yogurt consumption [14,15] have been associated with IGF-1-mediated acne pathogenesis [16,17]. Individuals with Laron syndrome, who exhibit a congenital IGF-1 deficiency, are of short stature and do never develop acne [18], unless therapeutically substituted with recombinant IGF-1 [19].

2.2. IGF-1-PI3K-AKT-Mediated Downregulation of FoxO1 and FoxO3

After binding of IGF-1 to its receptor, the phosphoinositide 3-kinase (PI3K) gets activated resulting in the activation of the kinase AKT (also known as protein kinase B), a key checkpoint of growth network regulation [20]. Among many regulatory effectors, AKT phosphorylates and thereby inactivates the transcription factors FoxO1 and FoxO3, predicted to play a key role in acne pathogenesis [21]. After AKT-mediated phosphorylation, nuclear FoxO1 is extruded into the cytoplasm, confirmed in IGF-1-stimulated SZ95 sebocytes *in vitro* [22]. FoxO1 acts as a negative nuclear coregulator of lipogenic transcription factors including androgen receptor (AR) [23], sterol regulatory element-binding transcription factor 1 (SREBF1) [24], peroxisome proliferator-activated receptor-γ (PPARγ) [25] and of the pro-inflammatory signal transducer and activator of transcription 3 (STAT3), respectively [26].

FoxO1 binds to STAT3 and prevents STAT3 from interacting with the SP1.POMC promoter complex, and consequently, inhibits STAT3-mediated leptin action. As shown in SZ95 sebocytes, leptin promotes a proinflammatory lipid profile and induces inflammatory pathways in human SZ95 sebocytes [27].

Thus, increased IGF-1 signaling via AKT-mediated suppression of FoxO1 enhances the transcriptional activity of AR, SREBF1, PPARγ and STAT3, crucial transcription factors promoting sebaceous lipogenesis [28–30]. In fact, IGF-1 has been shown to induce SREBF1 expression and lipogenesis in SEB-1 sebocytes via activation of the PI3K/AKT pathway [30], whereas a low glycemic diet, which decreases IGF-1 serum levels [12], reduces SREBF1 expression in sebaceous glands (SGs) of acne patients [31] (Figure 1).

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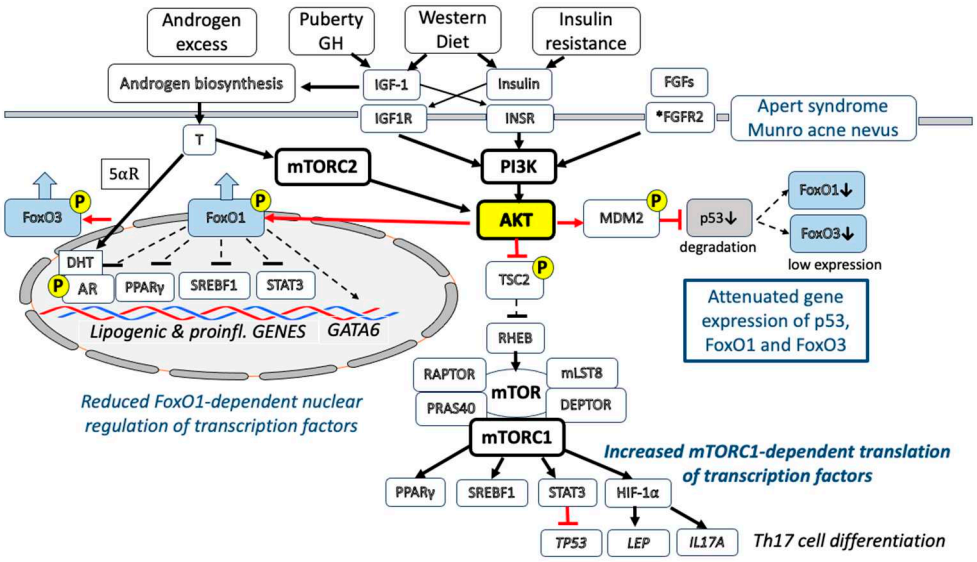

Figure 1. Disturbed transcriptional regulation in acne vulgaris. Growth factors and androgens overstimulate the kinase AKT (protein kinase B) in acne vulgaris. Increased puberty-mediated insulin-like growth factor 1 (IGF-1) signaling increases the activity of phosphoinositide 3-kinase (PI3K), which phosphorylates and activates AKT. Increased IGF-1/insulin signaling by Western diet (hyperglycemic carbohydrates, milk and dairy products) as well as fibroblast growth factor receptor 2 (FGFR2) gain-of-function mutations (Apert syndrome, Munro acne nevus) further augment the activation of AKT. IGF-1 stimulates adrenal and gonadal androgen biosynthesis and activates 5α-reductase (5α-R) converting testosterone (T) to dihydrotestosterone (DHT), the high affinity ligand of androgen receptor (AR). Androgens activate mechanistic target of rapamycin complex 2 (mTORC2), which also phosphorylates and activates AKT. Thus, IGF-1 and androgens maintain a synergistic crosstalk resulting in the activation of AKT. AKT-mediated phosphorylation of AR mediates its nuclear transfer. In contrast, AKT-mediated phosphorylation of the transcription factors forkhead box O1 (FoxO1) and forkhead box O3 (FoxO3) promotes their export from the nucleus into the cytoplasm reducing their nuclear activity. FoxO1 is a suppressive nuclear coregulator of AR, peroxisome proliferator-activated receptor gamma γ (PPARγ), sterol regulatory element-binding transcription factor 1 (SREBF1) and signal transducer and activator of transcription 3 (STAT3) but promotes the expression of GATA-binding protein 6 (GATA6), the key regulatory transcription factor of infundibular keratinocytes. Thus, loss of nuclear FoxO1 activity enhances the expression of lipogenic genes (activated by AR, PPARγ, SREBF1) and proinflammatory genes (activated by STAT3) and attenuates the expression of GATA6. AKT-mediated phosphorylation of mouse-double minute 2 (MDM2) enhances the degradation of the transcription factor p53 resulting in reduced p53-mediated expression of FoxO1, FoxO3 and other p53 target genes. AKT-mediated phosphorylation of tuberin (TSC2) reduces its negative impact on Ras homolog protein enriched in brain (RHEB), the key activator of mechanistic target of rapamycin complex 1 (mTORC1). Activated mTORC1 stimulates protein translation of the transcription factors PPARγ, SREBF1, STAT3 and hypoxia-inducible factor 1α (HIF-1α). STAT3 is a negative regulator of *TP53*, whereas HIF-1α stimulates the expression of leptin (*LEP*) and interleukin 17A (*IL17A*) and Th17 cell differentiation.

2.3. IGF-1-IGF1R-PI3K-AKT-MDM2-Mediated Downregulation of P53

IGF-1/IGF1R/PI3K/AKT activation inhibits the activity of p53 [32]. AKT activated by exogenous IGF-1 promotes the phosphorylation of the p53-binding protein mouse double minute 2 (MDM2). This phosphorylation increases the ability of MDM2 to degrade p53 [32]. MDM2 promotes cell survival and cell cycle progression by inhibiting p53. To regulate p53, MDM2 must gain nuclear entry, thereby diminishes cellular levels of p53, and decreases p53 transcriptional activity [32]. Phosphorylated MDM2 finally promotes the proteasomal degradation of p53 [33,34]. Thus, IGF-1-AKT signaling lowers the level of nuclear p53, recently suggested to play a key role in acne pathogenesis and treatment [2,35] (Figure 1).

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2.4. IGF-1-IGF1R-PI3K-AKT-Mediated Activation of mTORC1

Mechanistic target of rapamycin complex 1 (mTORC1) is a key growth factor-and nutrient-dependent regulatory kinase orchestrating cell proliferation and anabolism and inhibiting autophagy [36,37]. Activated AKT phosphorylates tuberin (TSC2). TSC2 is inactivated by AKT-dependent phosphorylation, which destabilizes TSC2 and disrupts its interaction with hamartin (TSC1) [38]. The TSC1-TSC2 (hamartin-tuberin) complex, through its GAP (GTPase-activating protein) activity towards the small G-protein RHEB (Ras homolog protein enriched in brain) is a critical negative regulator of mTORC1 [39]. Thus, AKT-mediated phosphorylation of TSC2 plays a key role for growth factor-stimulated mTORC1 activation, predicted to be of fundamental importance in acne pathogenesis [40,41]. Indeed, increased expression of mTOR and mTORC1 activation has been detected in the epidermis and SGs of acne patients [42–44]. Western diet over-stimulates insulin/IGF-1/AKT/mTORC1 signaling [45]. Recent evidence indicates that milk is a highly specialized signaling system of mammals promoting mTORC1-dependent translation for postnatal growth [46]. mTORC1 has a special impact in regulating protein translation of specific transcription factors involved in the pathogenesis of acne including SREBFs, PPARγ, STAT3 and hypoxia-inducible factor-1α (HIF-1α) [47]. Further evidence obtained in adipocytes underlines the role of mTORC1 in leptin biosynthesis at the level of translation [48] (Figure 1).

2.5. Insulin-INSR-PI3K-AKT-Mediated Activation of mTORC1

Acne has been associated with syndromes associated with insulin resistance, especially polycystic ovary syndrome (PCOS) [49], which results in hyperinsulinemia [50]. Insulin and IGF-1 are sister growth hormones and insulin has been shown to bind and activate both, insulin receptor (INSR) and IGF1R. Both INSR and IGF1R also elicit common downstream signaling with phosphorylation of a family of INSR substrates and activation of the PI3K-AKT-mTORC1 pathway [51]. Milk exhibits a high insulinemic index [52]. High intakes of milk, but not meat, increase serum insulin and insulin resistance as shown in 8-year-old boys [53]. Thus, milk consumption stimulates insulin and IGF-1 signaling [45], a meaningful mechanism to stimulate mTORC1-driven postnatal growth and anabolism [46]. In contrast, metformin therapy lowers insulin resistance [54] and thus reduces insulin signaling towards SGs. Mirdamadi et al. [22] convincingly demonstrated that both insulin and IGF-1 enhance the PI3K/AKT pathway in SZ95 sebocytes *in vitro*.

2.6. FGFR2-PI3K-AKT-Mediated Activation of mTORC1

Patients with congenital fibroblast growth factor receptor 2 (FGFR2) gain-of-function mutations (S252W or P253R FGFR2) - either germline (Apert syndrome) [55–58] or segmental somatic (acne nevus of Munro) [59–61] develop acne during puberty and respond to isotretinoin treatment [64–64]. FGFR2 is a tyrosine kinase receptor that like INSR or IGF1R activates multiple pathways including PI3K/AKT signaling [55]. Gain-of-function FGFR2 signaling thus adds on IGF1R and INSR signaling of puberty enhancing the AKT-mTORC1 pathway featuring the acne in Apert syndrome and acne nevus of Munro (Figure 1).

2.7. Androgen Receptor Signaling Converges with PI3K-Mediated Activation of AKT

Decades ago, acne was believed to be primarily an androgen-driven disease. Whereas the impact of androgens on acne pathogenesis is well accepted [2,28,65], recent observations underline a complex synergistic crosstalk between IGF-1, insulin and androgens in the activation of AKT and mTORC1. Androgens operate via ligand binding to AR. IGF-1 plays a critical role in the synthesis of adrenal and gonadal androgens [2,9,66–71] and promotes 5α-reductase-mediated conversion of testosterone to dihydro-testosterone (DHT) [72], the high affinity ligand of AR [73,74]. Androgens induce sebaceous differentiation in sebocytes expressing a stable functional AR [75]. Notably, two androgen response elements (AREs) have been identified in the upstream promoter of the *IGF1* gene [76]. It has been shown in prostate cancer cells that androgens also upregulate IGF1R expression [77]. DHT increases the association of mTOR with RICTOR instead of RAPTOR consistent with selective

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activation of mechanistic target of rapamycin complex 2 (mTORC2) by DHT [78]. Like PI3K, the growth promoting kinase mTORC2 phosphorylates and activates AKT and thereby contributes to nuclear extrusion of FoxO1 [78]. Thus, the insulin/IGF-1-PI3K signaling pathway and the androgen-mTORC2 signaling pathway converge in the activation of AKT (Figure 1). AKT-mediated nuclear extrusion of FoxO1, which is a negative coregulator of AR, further enhances the transcriptional activity of AR [23]. In addition, AKT phosphorylates AR enhancing AR transactivation and AR nuclear stability [79]. AKT-MDM2-mediated suppression of p53 [32–34] will further enhance AR signaling because AR expression is negatively regulated by p53 [80]. Notably, androgen-insensitive subjects who lack functional ARs do not produce sebum and do not develop acne [81] underlining the important contribution of AR signaling in the pathogenesis of acne.

3. Hypoxia-Inducible Factor-1a and Leptin

In 2014, Danby [82] postulated that ductal hypoxia may link comedogenesis and inflammation in acne. It has been hypothesized that ductal hypoxia as well as mTORC1 activation via upregulation of HIF-1a may enhance sebocyte-derived leptin secretion into the ascending sebum that may subsequently stimulate the proliferation of adjacent infundibular keratinocytes promoting comedogenesis [83]. The key transcription factor of hypoxia is HIF-1a [84]. HIF-1a translation is upregulated by activated mTORC1 [47].

Remarkably, HIF-1a transactivates the human leptin (*LEP*) gene promoter [85]. It has been shown in human keratinocytes that leptin mediates mitogenic stimuli promoting keratinocyte proliferation [86]. Primary keratinocytes under hypoxic conditions induce filaggrin (*FLG*) gene expression in a HIF-1α- and HIF-2α-dependent manner [87]. In fact, increased filaggrin expression has been observed in the sebaceous duct and infundibulum of patients with acne vulgaris [88]. The upregulation of the leptin-induced proinflammatory cytokines in normal human keratinocytes is mainly regulated by STAT3 signaling [89], which is also stimulated by overactivated mTORC1 [47].

HIF-1α acts via the retinoic acid receptor-related orphan receptor-γt (RORγt) to drive Th17 cell differentiation and is thus a key reprogrammer of metabolism in inflammatory cells promoting inflammatory gene expression [90]. HIF-1α controls the balance of Th17 cells and regulatory T cells [91]. Under conditions of hypoxia, most eukaryotic cells can shift their primary metabolic strategy from predominantly mitochondrial respiration towards increased glycolysis to maintain ATP levels [92]. At the transcriptional level, this metabolic switch is critically dependent on HIF-1α, which induces the expression of glycolytic enzymes. Glycolysis generates indispensable metabolic intermediates [93] that are required for the rapid proliferation of keratinocytes [94,95], sebocytes [96] and Th17 cells [97], thus linking comedogenesis and Th17 cell-mediated inflammation in acne [82,98]. A pilot study indicated that acne lesion extraction reduces HIF-1α expression [99]. Choi et al. [100] recently reported increased levels of SREBF1 and perilipin 2 (PLIN2) that were upregulated by HIF-1α in SZ95 sebocytes under hypoxia indicating that a hypoxic microenvironment can increase lipogenesis and provides a link between seborrhea and inflammation. However, we have to keep in mind that not only local hypoxia, but also increased mTORC1 signaling generally enhances the expression of HIF-1α [47] and leptin [48], respectively.

Surprisingly, there are no data on the effect of benzoyl peroxide, the most commonly used topical anti-acne agent, on ductal HIF-1α expression in acne skin. Li et al. [101] reported that minocycline, a commonly used oral antibiotic for the treatment of inflammatory acne [102,103], induces HIF-1α proteasomal degradation under hypoxia by increasing the expression prolyl hydroxylase-2 and HIF-1α/von Hippel-Lindau protein interaction, thereby overcoming hypoxia-induced HIF-1α stabilization. Azelaic acid, another topically used anti-acne agent, might also interfere with HIF-1α because it is known to inhibit anaerobic glycolysis [104]. An intensive crosstalk between p53 and HIF-1α as mediators of molecular responses to physiological and genotoxic stresses has been reported [105]. Activated p53 decreases HIF-1α protein levels by accelerated proteasome-dependent degradation [106]. In contrast, AKT activation inhibits p53-mediated HIF-1α degradation [107]. Remarkably, PI3K-AKT activation is required for the hypoxic stabilization of HIF-1α and hypoxia alone is not sufficient to render HIF-1α resistant to proteasomal cleavage and degradation

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[108]. Of note, we recently detected increased expression of HIF-1α in lesional skin of patients with acne inversa (hidradenitis suppurativa) [109].

4. Infundibular GATA-Binding Protein 6

Recent studies reported that the infundibulum and sebaceous ducts are lined by molecularly distinct differentiated cells expressing markers including keratin 79 and the transcription factor GATA-binding protein 6 (GATA6) [110–112]. Loss of GATA6 causes dilation of the hair follicle canal and sebaceous duct [111]. Importantly, Oulès et al. [112] observed reduced expression of GATA6 in the upper pilosebaceous unit in acne patients. GATA6 controls keratinocyte proliferation and differentiation to prevent hyper-keratinization of the infundibulum, which is the primary pathological event in acne vulgaris (comedogenesis). Okabe et al. [113] found the presence of retinoic acid response elements (RAREs) in the putative regulatory region of the *GATA6* gene and identified retinoic acid as a critical signal inducing GATA6 transcription. Topical treatment of acne with *all-trans* retinoic acid (ATRA) may thus normalize disturbed follicular keratinization promoting comedolysis via the induction of GATA6 expression. Importantly, the expression of GATA6 is also upregulated by FoxO1 [112]. *GATA6* exhibits three putative FoxO1 binding sites. Inhibition of AKT reduces phosphorylated FoxO1 and increases nuclear localization of FoxO1 subsequently enhancing GATA6 expression [114]. In contrast, overexpression of IGF-1/AKT signaling may thus reduce infundibular *GATA6* expression promoting comedogenesis via suppression of nuclear FoxO1 and GATA6. mTORC2 signaling, the androgen-driven pathway to activate AKT, also negatively regulates *GATA6* expression in a FoxO1-dependent manner [114]. Thus, increased insulin/IGF-1-AKT signaling as well as androgen-mTORC2-AKT signaling converge in the suppression of GATA6. Intriguingly, overexpression of GATA6 suppresses AR expression [110], pointing to enhanced infundibular AR signaling in reduced states of GATA6. Notably, inhibition of mTORC1 stabilizes GATA6 and promotes the nuclear accumulation of GATA6 [115]. In accordance with Oulès et al. [112], GATA6 expression contributes to the therapeutic effect of ATRA, the main topical treatment for comedonal acne. Isotretinoin/ATRA induced overexpression of p53 and FoxO1 with associated attenuation of PI3K-AKT-mTORC1 signaling may stabilize infundibular GATA6 in acne, a potential key mechanism of comedolysis. Isotretinoin treatment of human SEB-1 sebocytes induces cell cycle arrest associated with increased expression of p21 (cyclin-dependent kinase inhibitor 1A; *CDKN1A*) [116]. The *CDKN1A* gene is transcriptionally activated by p53 [117]. Remarkably, transient transfection with a GATA6 expression vector inhibited S-phase entry in vascular smooth muscle cells and in mouse embryonic fibroblasts lacking both p53 alleles. The GATA6-induced growth arrest correlated with a marked increase in the expression of p21 [118]. Thus, there appear to be a close interactions between isotretinoin-induced upregulation of p53-FoxO1, p53-p21 and FoxO1-GATA6, respectively. In contrast, hypoxia-induced microRNA-181b has been shown to target GATA6 [119], thus eventually destabilizing GATA6-controlled infundibular keratinocyte homeostasis linking ductal hypoxia to ductal GATA6 deficiency in acne.

5. Transforming Growth Factor β

Genome-wide associations studies identified gene loci associated with impaired transforming growth factor β (TGFβ) signaling including the genes *TGFB2*, *OVOL1* and *FST* [120,121]. In fact, experimental evidence supports the view that TGFβ signaling is reduced in SGs of acne patients [112]. Activation of the TGFβ signaling pathway is necessary and sufficient for maintaining sebocytes in an undifferentiated state. The presence of TGFβ suppresses genes required for the production of sebaceous lipids and for sebocyte differentiation such as fatty acid desaturase 2 (FADS2) and PPARγ, thereby decreasing lipid accumulation through a TGFβR2-SMAD2 dependent pathway [122]. There is an important molecular crosstalk between GATA6 and TGFβ. GATA6 is a critical signal for the activation of TGFβ [113]. In a sebaceous organoid model, Oulès et al. [112] obtained evidence that GATA6-mediated TGFβ activation is a key process controlling the repression of the interfollicular fate of keratinocytes and promoting junctional zone/sebaceous duct differentiation and downregulation of the infundibular differentiation program [112]. Furthermore, it has been shown

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in chondrocytes that TGFβ stimulates FoxO1 expression [123] pointing to a crosstalk of GATA6, TGFβ and FoxO1, respectively.

6. Cutibacterium acnes

Obstruction of the pilosebaceous unit leads to hypoxia, favoring the development of *Cutibacterium acnes* (*C. acnes*), formerly designated as *Propionibacterium acnes* (*P. acnes*). Multiple studies suggest that dysbiosis of the skin microbiota significantly contributes to acne development. *C. acnes* is the dominant resident bacterial species in SG-enriched areas of the skin implicated in acne pathogenesis and disease progression [124]. Acne has been linked to dysregulated innate immunity in response to *C. acnes* promoting increased Th17 cell differentiation with increased IL-17 levels [125], thus contributing to sebocyte-mediated polarization of cutaneous T cells towards the Th17 phenotype [126]. To mimic the effect of *C. acnes* on sebocytes, Oulès et al. [112] treated control or GATA6-expressing sebocytes with peptidoglycan (PGN), which is the main component of the Gram+ bacterial cell wall, which is recognized by toll-like receptor 2 (TLR2). GATA6 expression led to a decrease in PGN-induced IL-17 expression. When they explored the effect of live *C. acnes* bacteria on control and GATA6 expressing sebocytes, GATA6 expression showed a trend towards deceased IL-17 expression [112]. It has been reported that *C. acnes* membrane fractions increase IGF-1 and IGF1R expression in the epidermis of explants [127], a constellation that may reduce FoxO1-mediated GATA6 expression. *C. acnes* also induces TLR2 and TLR4 on keratinocytes, a mechanism that could play an essential role in acne-linked inflammation [128], as TLR2 activation drives an inflammatory transcriptional program in Th17 cells [129]. Biofilm *C. acnes* in contrast to planktonic bacteria are characterized by upregulated stress-induced genes and upregulation of genes coding for the potential virulence-associated CAMP factors [130] including exogeneous triacylglycerol lipase (*gehA*) [131] enhancing the release of free fatty acids like palmitic and oleic acid [132,133]. Lipase-mediated triacylglycerol hydrolysis thus generates saturated free fatty acids that bind and activate TLR2-triggered proinflammatory signaling [134]. Gene expression of interleukin 8 (IL-8) and TLR2 was enhanced by cell-free extracts of *C. acnes* in SZ95 sebocytes [135]. Palmitic acid can stimulate IL-6, TNF-α, IL-1β productions in HaCaT keratinocytes promoting cell proliferation, potentially contributing to inflammation and pilosebaceous duct hyperkeratinization in acne [136].

Taken together, *C. acnes*-derived signaling augments a variety of transcriptomic deviations observed in acne vulgaris.

7. Transcriptomic Effects of Isotretinoin Treatment

Isotretinoin counteracts critical pathways of growth factor and androgen signaling that are overstimulated in acne vulgaris.

7.1. P53/FoxO1-Mediated Suppression of IGF-1/IGF1R/PI3K/AKT/mTORC1 Signaling

Oral isotretinoin is the most effective treatment of recalcitrant and severe acne improving all major aspects of acne pathogenesis [137,138]. It has been predicted that isotretinoin upregulates the expression of FoxO1 and FoxO3 [139–141]. In the meantime, experimental evidence confirmed that isotretinoin enhances the expression of FoxO1 in primary human keratinocytes [142], SZ95 immortalized human sebocytes [143] and SGs of isotretinoin-treated acne patients [144]. We could demonstrate by immunohistochemistry of SGs that isotretinoin treatment increased nuclear accumulation of FoxO1 and FoxO3 proteins [144] pointing to changes of FoxO-mediated gene expression or FoxO translocation. Furthermore, it has been hypothesized that isotretinoin upregulates p53 in the pilosebaceous unit [35]. Notably, p53 maintains baseline expression of *FOXO1A* [145]. Importantly, *FOXO3A* has also been confirmed to be target gene of p53 [146]. Experimental evidence confirmed that isotretinoin treatment of primary human keratinocytes increased the expression of p53 [142]. Recently, we could demonstrate that isotretinoin treatment enhanced nuclear accumulation of p53 in SGs of acne patients [147]. Of note, prior to isotretinoin

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treatment, acne patients exhibited lower cutaneous expression levels of p53 protein compared to acne-free controls [147].

p53, FoxO1 and FoxO3 are fundamental negative regulators of cell cycle progression and cell proliferation. These proapoptotic transcription factors exert synergistic effects in promoting catabolism, autophagy and apoptosis, respectively [148–154] (Figure 2).

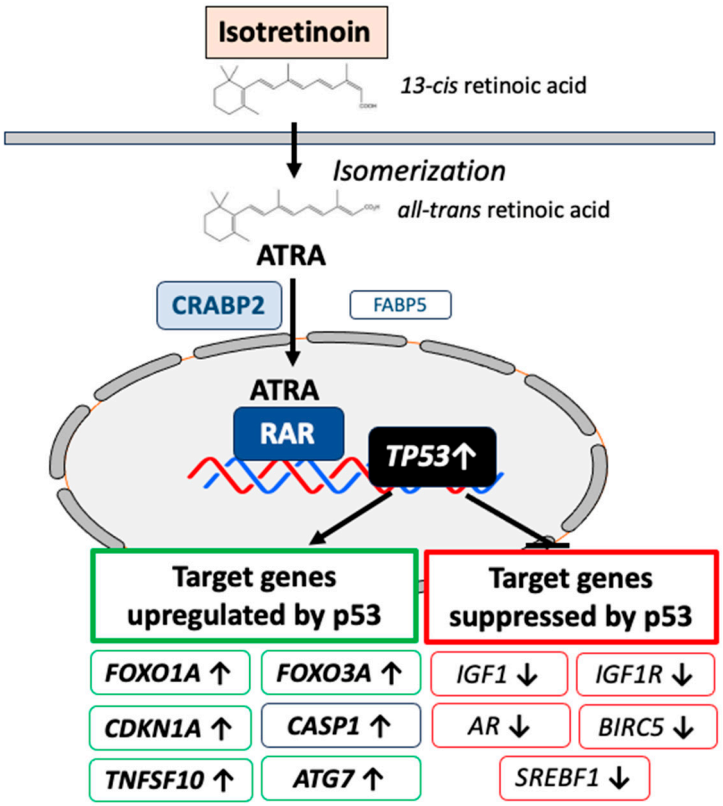

Figure 2. Isotretinoin-induced upregulation of p53. After cellular uptake of isotretinoin, *13-cis* retinoic acid is isomerized to *all-trans* retinoic acid (ATRA). The further transport of ATRA depends on the cellular abundance of cellular retinoic acid binding protein 2 (CRABP2), which in contrast to fatty acid binding protein 5 (FABP5) transfers ATRA to retinoic acid receptors (RAR) promoting the expression p53. p53 upregulates target genes promoting cell cycle arrest (*CDKN1A*), autophagy (*ATG7*) and apoptosis (*FOXO1A*, *FOXO3A*, *CASP1*, *TNFSF10*) but suppresses genes involved in growth factor signaling (*IGF1*, *IGF1R*), androgen signaling (*AR*), cell survival (*BIRC5*) and lipid biosynthesis (*SREBF1*), respectively.

p53 suppresses IGF-1/PI3K/AKT/mTORC1 activity at multiple regulatory checkpoints [155–164]. The *IGF1* promoter is negatively regulated by p53 [159]. In fact, Karadag et al. [160] observed decreased serum levels of IGF-1 in isotretinoin-treated acne patients. In addition, *IGF1R* expression is also negatively regulated by p53 [161]. Disruption of endogenous IGF1R led to inhibition of insulin receptor (*INSR*) promoter activity by p53 [162]. Of note, the expression of negative regulators of the PI3K-AKT-mTORC1 signaling pathway including phosphatase and tensin homolog (*PTEN*) (a negative regulator of PI3K), *TSC2* (negative regulator of RHEB), AMP-activated protein kinase β1 (*PRKAB1*) (activator of TSC2) are all upregulated by p53 [163]. DEP-domain containing mTOR-interacting protein (DEPTOR) is a natural inhibitor of mTORC1 and mTORC2. *DEPTOR* as well is a downstream target p53, whose activity positively correlates with DEPTOR expression [165].

Taken together, isotretinoin-mediated upregulation of p53 counteracts multiple regulatory checkpoints of exaggerated IGF-1/IGF1R/PI3K/AKT-mTORC1 and potentially mTORC2 signaling pathways in acne patients (Figure 3).

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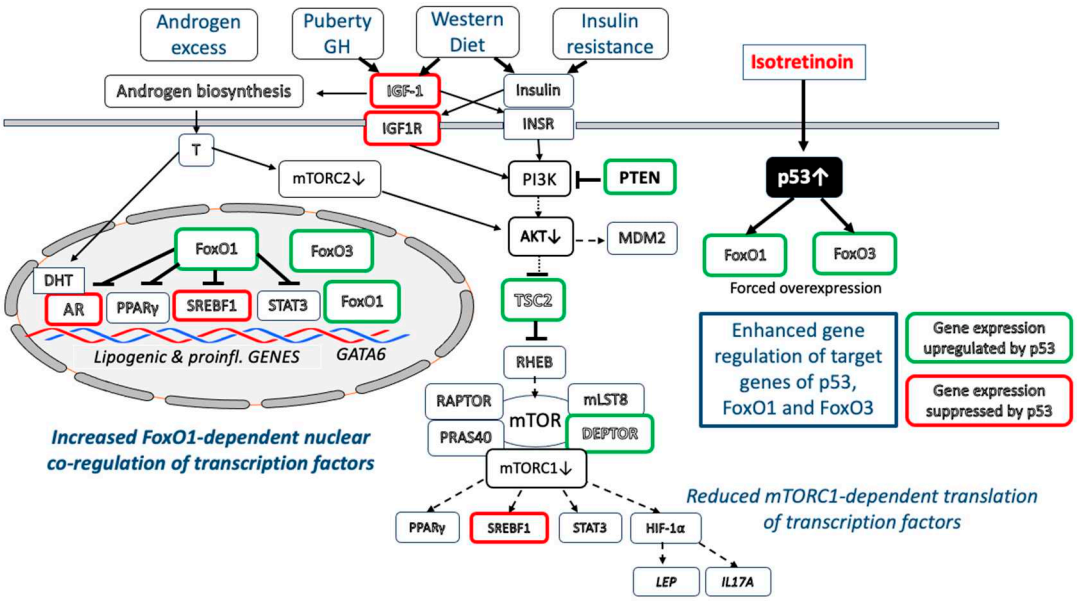

Figure 3. Isotretinoin-induced overexpression of p53 counteracts dysregulated transcriptomics of acne vulgaris. Reduced gene expression of insulin-like growth factor 1 (*IGF1*) and insulin-like growth factor 1 receptor (*IGF1R*) reduces the activation of phosphoinositide 3-kinase (PI3K), which is further suppressed by upregulated expression of phosphatase and tensin homolog (*PTEN*). Reduced IGF-1/IGF1R signaling also attenuates androgen biosynthesis resulting in reduced mTORC2-mediated activation of AKT. Increased expression of tuberin (*TSC2*) suppresses Ras homolog protein enriched in brain (RHEB), thereby reduces the activity of mTORC1. mTORC1 and mTORC2 are further inhibited by induced expression of DEP-domain containing mTOR-interacting protein (*DEPTOR*), a natural inhibitor of both mTORC1 and mTORC2. Reduced mTORC1 results in impaired protein translation of peroxisome proliferator activated receptor γ (PPARγ), sterol regulatory element-binding transcription factor 1 (SREBF1), signal transducer and activator of transcription 3 (STAT3) and hypoxia-inducible factor 1α (HIF-1α). Forced nuclear expression of FoxO1 inhibits the transcriptional activity of androgen receptor (AR), PPARγ, SREBF1, STAT3 but induces the expression of *GATA6*. Thus, isotretinoin suppresses overactive growth factor and androgen signaling in acne patients but enhances GATA6 signaling, the key transcription factor controlling infundibular homeostasis. Forced overexpression of p53, FoxO1 and FoxO3 augments proapoptotic signaling explaining isotretinoin´s desired pharmacological mode of action (sebum suppression via sebocyte apoptosis) but also its major adverse effects, especially its teratogenicity (neural crest cell apoptosis).

7.2. P53/FoxO1 Upregulation Suppresses AR Signaling

In a murine model, Cottle et al. [166] demonstrated that p53 activation inhibits SG differentiation and disrupts AR signaling. It has been shown that upregulation of p53 inhibits AR expression [80,167]. In fact, oral isotretinoin treatment reduced AR levels in the skin of male acne patients [168].

Thus, isotretinoin attenuates IGF-1/IGF1R and AR signaling, two critical converging pathways involved in AKT/mTORC1-driven acne pathogenesis [169] (Figure 3).

7.3. GATA6 Upregulation Suppresses Comedogenesis

Reduced infundibular *GATA6* expression has been related to disturbed follicular keratinization (comedo formation) in acne patients [112]. Systemic isotretinoin treatment has a two principal impacts on the upregulation of infundibular *GATA6* expression: 1) After isomerization of isotretinoin (*13-cis* retinoic acid) to ATRA [170], ATRA activates RAR [171] enhancing GATA6 expression via RAR binding to RARE on the *GATA6* promoter [113]. In addition, p53-mediated upregulation of nuclear FoxO1 may activate three FoxO1 binding sites on the *GATA6* promoter [112,114] further

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promoting its expression. Upregulated GATA6 represses AR activation [110], a synergistic interplay normalizing disturbed infundibular keratinization (comedogenesis) in acne patients.

7.4. Sebum Suppression, Sebocyte Autophagy and Apoptosis

It is well accepted that binding of ATRA initiates changes in interactions of RAR/RXRs with co-repressor and co-activator proteins, activating transcription of primary target genes, alters interactions with proteins that induce epigenetic changes and induces transcription of genes encoding transcription factors and signaling proteins that further modify gene expression [172]. Isotretinoin-induced upregulation of p53 explains sebocyte apoptosis and sebum suppression as the major desired pharmacological effects in the treatment of acne [35,173]. Nelson et al. [174] demonstrated that tumor necrosis factor-related apoptosis inducing ligand (TRAIL) contributes to the apoptotic effect of isotretinoin in human sebocytes. Importantly, p53 upregulates the expression of the proapoptotic proteins TRAIL (*TNFSF10*) [175], FoxO1 (*FOXO1A*)[144,145] and FoxO3 (*FOXO3A*)[144], respectively. In contrast, survivin (*BIRC5*), a member of the inhibitors of the apoptosis gene family, which is overexpressed in serum of acne patients [176,177], is suppressed by p53 [178]. Thus, isotretinoin-mediated upregulation of nuclear p53 in SGs [147] represents most likely the desired sebum-suppressive effect in promoting sebocyte apoptosis. In SGs, isotretinoin (*13-cis* retinoic acid) is converted to *all-trans* retinoic acid (ATRA) [170]. Via binding to cellular retinoic acid binding protein 2 (CRABP2), ATRA is transported into the nucleus, where in a RAR-dependent fashion the transcription of p53 is upregulated [179]. ATRA/RAR-mediated upregulation of p53 expression has also been reported in other benign and malignant cells [180–185]. Preferential CRABP2 expression in basal and suprabasal layers of SGs is mandatory for ATRA-induced transcriptomic changes resulting in sufficient sebum suppression [186]. It is of critical functional importance that partitioning of ATRA between the nuclear receptors RAR and PPARβ/δ is regulated by the intracellular lipid binding proteins CRABP2 and fatty acid binding protein 5 (FABP5) [187]. In cells with a high CRABP2/FABP5 ratio, ATRA functions through RAR and is a proapoptotic agent, but in cells that highly express FABP5 it signals through PPARβ/δ and promotes cell survival [187]. Increased expression of CRABP2 in isotretinoin-treated SGs of patients with acne has been observed after weeks of oral isotretinoin exposure [186], whereas short-term (6 h, 24 h) isotretinoin exposure of immortalized SZ95 sebocytes did neither exhibit increased CRABP2 nor upregulated p53, FoxO1 or FoxO3 expression [188,189].

Recently, Seo et al. [143] reported that autophagy is constitutively active in maturing sebocytes of human SGs, whereas autophagy-related protein expression is repressed in SGs of acne patients. The authors found that isotretinoin activates autophagy in immortalized SZ95 sebocytes and induces sebosuppression [143]. Blocking autophagy using siRNA targeting autophagy-related 7 (ATG7) resulted in a significant loss of the sebosuppressive activity of isotretinoin in SZ95 sebocytes stimulated with testosterone and linoleic acid. Unfortunately, the investigators overlooked the reported intimate interaction between ATG7 and p53. ATG7 binding to p53 enhances the transcription of the cell cycle inhibitor p21 [190]. Among the genes directly activated by p53 are several autophagy genes, including ATG7 [191]. It is well established that there is an important relationship between autophagy and p53. Autophagy suppresses p53, whereas p53 also activates autophagy [192]. In this regard, studying autophagy in immortalized SZ95 sebocytes is critical because Simian virus transfection used for sebocyte immortalization is mediated by inactivation of p53 by direct binding of SV40 large T antigen to p53 [193,194]. Proapoptotic p53 signaling may thus be compromised in p53-inactivated immortalized sebocytes, which may thus be unable to execute to complete program of p53-induced apoptosis but instead remain in a preliminary state of autophagy. In contrast, human sebocytes *in vivo* are not artificially p53-inactivated and the clinical period to obtain an histological "involution" of SGs in acne patients takes weeks of isotretinoin treatment compared to short term cell culture studies [195].

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7.5. Teratogenicity and Neural Crest Cell Apoptosis

Neural crest cells (NCCs) and neural crest (NC)-derived neuroblastoma cells are very susceptible to isotretinoin-induced apoptosis [173,196,197]. Neural precursor cells possess multiple p53-dependent apoptotic pathways [198]. Translational evidence suggests that isotretinoin-induced upregulation of p53 promotes NCC apoptosis suggested to operate as the major pathogenic mechanism of isotretinoin´s teratogenicity [199]. Increased p53 signaling is also associated with Treacher Collins-, CHARGE- and fetal alcohol syndrome, which all exhibit dysmorphic craniofacial features resembling retinoid embryopathy [199]. It has been demonstrated in mouse embryonic fibroblasts that p53 controls the NC/EMT gene network [200]. Upregulation or stabilization of p53 in the cranial neural tube reduces cranial NC delamination and promotes neural tube defects in chick embryos [200]. p53 was shown to play a major role in Treacher Collins syndrome, a congenital haploinsufficiency disorder in humans that arises from mutations in the *TCOF1* gene. In the absence of one *Tcof1* allele in the mouse, upregulation of p53-related apoptotic genes in NC progenitors results in severe craniofacial defects [201]. Keeping in mind that pathogenic mechanisms involved in Treacher Collins syndrome are dependent on the p53 pathway, recent *in vivo* ATRA-treatment results showed similar processes where ATRA-exposure led to an increase of apoptosis processes at late time points and thus could participate in producing developmental birth defects [202]. Using a proteomic approach, a new ATRA target, *EFTUD2* (elongation factor Tu GTP-binding domain-containing 2) has been identified whose dysregulation leads to craniofacial defects [202]. Homozygous deletion of *Eftud2* causes brain and craniofacial malformations, affecting the same precursors as in mandibulofacial dysostosis with microcephaly patients [203]. Remarkably, increased p53 activity and NCC death are responsible for craniofacial malformations in the *Eftud2; Wnt1-Cre2* mutant mouse model [203]. Overactivation of the p53 pathway in *Eftud2* knockdown cells was attenuated by overexpression of non-spliced MDM2, and craniofacial development was improved when *Eftud2*-mutant embryos were treated with pifithrin-α, an inhibitor of p53 [203].

Thus, accumulating evidence underlines the role of overactivated p53 signaling in NCC apoptosis linking syndromes with dysmorphic craniofacial features with the pathogenesis of isotretinoin-induced embryopathy (teratogenicity).

7.6. Depression and Impaired Hippocampal Neurogenesis

The question whether isotretinoin causes depression and anxiety in acne patients is still a matter of debate [204–209]. However, a subgroup of patients may be at risk of developing depression or suicidal ideation with isotretinoin treatment [205,208]. The hippocampus is one of the brain regions where new neurons are constantly formed in a process called hippocampal neurogenesis [210–213]. Reduced hippocampal volume and low numbers of hippocampal neural progenitors have been reported in depressed humans [213]. The commonly used anti-depressive drug lithium has been shown to increase hippocampal neurogenesis in rodents and humans [214–217]. Long-term lithium therapy has been shown to suppress neuronal p53 levels [218,219] and reduces FoxO3 transcriptional activity by decreasing its intracellular content [220]. Intriguingly, acne is a possible adverse effect of lithium therapy [221–223], a potential result of attenuated p53/FoxO signaling. Remarkably, isotretinoin treatment of mice results in both decreased hippocampal neurogenesis and reduction in hippocampal volume [224,225]. Treatment of hypothalamic cells with 10 μM isotretinoin for 48 h decreased cell growth to 45.6 $\pm$ 13% of control [225]. Griffin et al. [225] hypothesized that the ability of isotretinoin to decrease hypothalamic cell numbers may contribute to the increased depression-related behaviors observed in mice. Intracerebroventricularly applied ATRA to adult rats increased RARα protein expression in the hippocampus suggesting an activation of ATRA/RARα-induced signaling mechanisms [226]. In these rats, ATRA-induced impairments in hippocampal neurogenesis correlated with depression-like symptoms [226]. Remarkably, retinoic acid-inducible gene 1 (*RAI1*) was found to be significantly upregulated in brains from patients with schizophrenia, bipolar disorder, or major depression [227]. Gene expression profiling reveals the role of RAI1 like receptor signaling in p53 dependent apoptosis induced by psoralen + UV-A (PUVA) in keratinocytes [228]. A direct relationship between p53 expression and loss of viability in CNS neurons [229] and neuronal

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cell death is well established [230–232]. In fact, adenovirus-mediated delivery of the p53 gene causes cortical and hippocampal neuronal cell death with some features typical of apoptosis [233].

Recent studies have shown that FoxOs are as well implicated in the pathophysiology of depression [234,235]. They play an essential role for neural stem cell homeostasis [236]. In gerbil and mouse brains dephosphorylation of FoxO1 following transient forebrain ischemia resulted in FoxO1 translocation into the nucleus in neurons [237]. The activation of FoxO1 preceded delayed neuronal death in the vulnerable hippocampal regions following ischemic brain injury. Notably, FoxO1 activation was accompanied by an increase in DNA binding activity for FoxO1-responsive element on the Fas ligand promoter (*FASLG*) [237]. In accordance to AKT-mediated regulation of FoxO1, AKT-induced phosphorylation of FoxO3 promotes cell survival via extrusion of FoxO3 from the nucleus into the cytoplasm [238,239], whereas nuclear non-phosphorylated FoxO3 triggers apoptosis inducing Fas ligand (*FASLG*) expression [239]. Of note, *FOXO1A* is a target of FoxO3, which induces the expression of FoxO1 [240]. IGF-1-induced AKT-mediated phosphorylation of FoxO3 has been observed in NC-derived PC12 cells, where IGF-1 inhibits apoptosis of neuronal cells [241]. Studies in murine models confirmed that FoxO1 and FoxO3 influence behavioral processes linked to anxiety and depression [242]. In fact, IGF-1-PI3K-AKT-mediated phosphorylation of FoxO3 induces survival of cultured hippocampal neurons [243]. In contrast, overexpression of FoxO3 induces apoptosis of cultured hippocampal neurons [243]. In this regard, it is of critical concern that we observed nuclear overexpression of both FoxO1 and FoxO3 in SGs of acne patients treated with isotretinoin [144], a pharmacologically constellation that may negatively affect the homeostasis hypothalamic neurons. In accordance, Kim et al. [244] showed that overexpression of FoxO1 suppresses neuronal differentiation, whereas in the absence of FoxO-dependent homeostatic processes, there was significant decrease in the neuronal stem cell pool and accompanying neurogenesis in the adult mouse brain.

Thus, isotretinoin-mediated upregulation of p53, FoxO1 and FoxO3 might negatively affect hypothalamic neurogenesis eventually increasing the risk of depression in predisposed individuals.

7.7. Reduced Ovarian Reserve and Granulosa Cell Apoptosis

Systemic isotretinoin also modifies the pituitary-ovarian axis causing mild suppression of pituitary hormone levels including growth hormone (GH), thyroid stimulating hormone (TSH), prolactin, adrenocorticotropic hormone (ACTH), and luteinizing hormone (LH) [245,246]. Follicle-stimulating hormone (FSH) and LH are required for the maturation of ovarian follicles. FoxO1 is critically involved in the regulation of gonadotropin expression [247–250]. Increased nuclear localization of FoxO1 decreases FSH β-polypeptide (*FSHB*) mRNA levels in murine primary pituitary cells [248]. FoxO1 overexpression in pituitary gonadotrope cells also inhibits the transcription of the β-subunit of LH (*LHB*) [249].

In rats, isotretinoin reduced ovarian reserve, a process associated with granulosa cell (GC) apoptosis [251]. In women treated with isotretinoin, anti-Müllerian hormone, antral follicle count and ovarian volume were significantly reduced compared to pretreatment values pointing to negative effects on ovarian reserve [252]. FoxO1 and FoxO3 are highly expressed in GCs of ovarian follicles. They interact with activin to regulate genes controlling follicle growth or with bone morphogenic protein 2 (BMP2) to control genes associated with metabolic stress and apoptosis leading to follicle death [253]. Overexpression of FoxO1 inhibits the viability of GCs in the mouse [254]. Constitutive activation of FoxO1 in murine GCs not only abolishes the protection from FSH, but activates autophagic gene expression [255]. Further experimental evidence in mice and chicken supports a critical role of FoxO1 and FoxO3 in GC apoptosis [256,257]. In addition, FSH and FoxO1 regulate genes in the sterol/steroid and lipid biosynthetic pathways in GCs [258]. It has been shown in porcine GCs that p53 promotes apoptosis and suppresses cell proliferation [259].

The impact of isotretinoin action on the pituitary-ovarian axis and GCs might as well be caused by overexpressed p53/FoxO1/FoxO3 signaling resulting in gonadotropin suppression and GC apoptosis, respectively [260].

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7.8. Hypertriglyceridemia

Isotretinoin-mediated upregulation of p53 and FoxO1 also provides a reasonable explanation for isotretinoin-induced hyperlipidemia, which results primarily from hepatic oversecretion of apolipoprotein B100-containing very low density-lipoproteins (VLDL) [261,262]. Gustafson et al. [263] showed that rats respond with a prompt increase (+ 250%) in plasma triacylglycerol levels within the first few days after starting the administration of isotretinoin, which was exclusively due to a marked increase in VLDL. We observed a significant increase in VLDL apolipoprotein B (apoB) levels during isotretinoin treatment of acne patients [262]. The hepatic synthesis of apo B (B-100) is absolutely required for VLDL assembly and secretion. It contains several very hydrophobic areas that serve as strong lipid-binding domains [264,265]. Intriguingly, the *APOB* gene has been identified as a target gene of p53 [266]. Loading of hepatic triglycerides onto apoB100 is mediated by microsomal triglyceride transfer protein (MTTP), which is also essential for VLDL assembly and secretion and is induced by FoxO1 [267]. In addition, FoxO1 stimulates hepatic apolipoprotein C-III (*APOC3*) expression. FoxO1 binds to its consensus site in the *APOC3* promoter [268,269]. In men, isotretinoin treatment (80 mg/d; 5 d) resulted in elevated plasma apoC-III concentrations at the transcriptional levels [270]. It has been shown that apoC-III strongly inhibits hepatic uptake of VLDL and intermediate density lipoproteins (IDL) [271] and inhibits the activity of lipoprotein lipase [272,273].

Thus, isotretinoin-mediated changes with transcriptional upregulation of p53 and FoxO1 explain isotretinoin-induced hypertriglyceridemia by increased hepatic VLDL apo B synthesis, triglyceride loading onto VLDL, heapticVLDL secretion but impaired VLDL triglyceride hydrolysis and hepatic VLDL and IDL uptake.

7.9. Increased Transepidermal Water Loss and Dry Skin

Dry skin is the most common mucocutaneous side effect of oral isotretinoin treatment [274]. Increased transepidermal water loss induced by oral isotretinoin treatment has been related to upregulated expression of aquaporin 3 (*AQP3*) [275]. AQP3 controls a water-, glycerol-, and hydrogen peroxide-transporting channel that plays a key role in various processes involved in keratinocyte function [276]. *AQP3* is another p53 target gene [277].

Isotretinoin-induced p53 overexpression in epidermal keratinocytes [142] thus explains increased AQP3-mediated transepidermal water loss resulting in dry skin.

7.10. Intracranial Hypertension

Intracranial hypertension (*pseudotumor cerebri*) is a potential adverse effect of oral isotretinoin treatment [278,279]. An association between increased choroid plexus (ChP) aquaporin 1 (*AQP1*) and intracranial pressure has also been observed in obese Zucker rats [280]. AQP1 plays an essential role in water movement through ChP epithelial cells. Of note, CRABP2 is specifically expressed in ChP [281]. AQP1 is primarily located in the apical membrane of the ChP epithelium and has been implicated in playing a pivotal role in cerebrospinal fluid secretion [282]. AQP1 is widely distributed in the human brain and is associated with water secretion into the subarachnoid space and has been suggested to play a key role in idiopathic and drug-induced intracranial hypertension [283]. A close correlation between upregulated expression of p53, p21 and AQP1 has been reported in rat kidney, heart, lung and small intestine [284]. In fact, a recent bioinformatic analysis of the *AQP1* promoter revealed the presence of DNA binding sites for p53 [285].

Thus, isotretinoin-induced overexpression of p53 in ChP epithelial cells may explain the occurrence of intracranial hypertension in predisposed individuals.

7.11. Inflammatory Flare at Initiation of Isotretinoin Treatment

Interleukin 1β (*IL1B*) mRNA and the active processed form of IL-1β are abundant in inflammatory acne lesions [286–288]. Caspase 1, also known as IL-1 converting enzyme, is a protease responsible for the processing of the key proinflammatory cytokine IL-1β from the inactive precursor pro-IL-1β to the active, secreted IL-1β [289,290]. In addition to the processing of IL-1β, caspase-1 plays

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an important and a conserved role as a cell death protease [288]. Remarkably, *CASP1* is a target gene of p53 [291]. Thus, initiation of isotretinoin therapy with upregulation of p53 might explain the inflammatory acne flare at the beginning of isotretinoin treatment [292] and the occurrence of acne fulminans after initiation of isotretinoin therapy [293,294]. Growing evidence suggests that many of the signaling molecules known to regulate programmed cell death can also modulate inflammasome activation in a cell-intrinsic manner [295].

8. Limitations of Immortalized Human Sebocytes

In 1979, p53 was first identified in complex with the SV40 tumor-virus oncoprotein [296–299]. The immortalized human sebocyte cell lines SZ95 [300] and SEB-1 [301] were generated in 1999 and 2001, respectively. They are widely used tools for acne research and the exploration of pharmaceutical effects of potential anti-acne agents. Both immortalized sebocyte cell lines are generated by Simian virus (SV) transfection [300,301]. Importantly, SV40 large T antigen binds and inactivates p53 [193,194]. Furthermore, it has been shown that SV40 T antigen inhibits binding of p53 to cellular DNA [302]. As p53, called the guardian of the genome [303], plays key roles in the regulation of lipid metabolism [304–309], cell cycle control [310,311], autophagy [192] and apoptosis [312–314], viral inactivation of p53 by SV40 transfection will certainly cause deviations from normal physiological and pharmacological cellular responses compared to primary human non-immortalized sebocytes or *in vivo* SG specimens obtained from human subjects, in whom p53 is not artificially inactivated. Although an immortalized cell should generally be resistant against cell death, Wróbel et al. [315] reported apoptosis in human immortalized SZ95 sebocytes after 6 h of SZ95 sebocyte treatment with high staurosporine concentrations (10^{-6}-10^{-5} M). However, treatment with *13-cis* retinoic acid (10^{-8}-10^{-5} M) did not affect externalized phosphatidylserine levels, DNA fragmentation, and lactate dehydrogenase cell release, despite increased caspase 3 levels leading to the conclusion that isotretinoin (*13-cis* retinoic acid) did not execute programmed death in human SZ95 sebocytes [315].

Under today´s perception, these former experiments require a critical re-evaluation: p53-inactivated SZ95 sebocytes apparently fail to induce a sufficient isotretinoin-mediated upregulation of p53 resulting in the incomplete execution of apoptosis. In addition, immortalized sebocytes treated with isotretinoin exhibit peculiar aberrations in the regulation of lipid metabolism. Short-term exposure of human SEB-1 sebocytes to *13-cis* retinoic acid (10^{-7} M, 10^{-6} M and 10^{-5} M) for 48 hours did not exhibit apoptosis, but paradoxically induced a dose-dependent increase in total lipid synthesis, SREBF1 and 5-lipoxigenase (ALOX5) expression [188]. Of note, it has been reported that SV40 large T antigen/p53 complex activates the promoter of the *IGF1* gene [316]. The isotretinoin-induced upregulation of p53 in SEB-1 cells may thus have increased the formation of SV40 large T antigen/p53 complex enhancing IGF-1-AKT-SREBF1 signaling enhancing lipogenesis [317]. A partial increase in isotretinoin-mediated upregulation of p53 in SEB-1 sebocytes may also explain the observed upregulation of *ALOX5*, a further target gene of p53 [318]. *SREBF1* expression is under physiological conditions negatively regulated by p53 [309] pointing to insufficient isotretinoin/p53-mediated SREBF1 suppression in immortalized SEB-1 sebocytes. Also, the statement that "isotretinoin is indirectly effective in sebocytes" [189], requires a re-evaluation. Observed metabolic effects in immortalized p53-inactived sebocytes should not be transferred to responses of primary human sebocytes, which have never been used as physiological controls in the majority of experiments with immortalized sebocytes. However, SGs of patients treated with isotretinoin *in vivo* exhibit significant nuclear upregulation of p53 [147], marked sebum reduction [319] and SG involution [320], most likely driven by p53-mediated apoptosis [117,173,174]. Immortalized sebocytes apparently mostly respond reasonably when studied under proliferative stimuli like the exposure of IGF-1 and insulin, but have an overlooked handicap with proapoptotic signaling, especially isotretinoin-regulated transcription.

The final physiological fate of sebocytes in SGs is holocrine secretion [321], a unique DNase2-dependent mode of programmed cell death [322]. Notably, during *Drosophila* embryonic development, cell death eliminates 30% of the primordial germ cells. Therefore, an intrinsic alternative cell death pathway is operative and mediated by DNase2 release from lysosomes, leading to nuclear translocation and subsequent DNA double-strand breaks [323,324]. Notably, nuclear

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accumulation of DNase2 requires binding to the mitochondrial protein apoptosis inducing factor (*AIFM1*), which is upregulated by p53 [325]. This sheds a new light on the mode of isotretinoin-treatment, which via upregulation of p53 may accelerate AIFM1/DNase2-dependend programmed cell death, a phenomenon, which cannot be followed in p53-inactivated immortalized sebocytes. It is thus important for future research, to focus on the transcriptomics of death pathways to get deeper insights for the understanding of sebocyte biology as well as therapeutic responses [326]. Table 1 and Table 2 summarize p53- and FoxO1-controlled target genes, which are critically involved in acne pathogenesis and isotretinoin´s mode of action.

Table 1. p53 target genes, whose expression is either down- or up-regulated by p53.

Gene	Gene name	References
AR	Androgen receptor ↓	[80]
IGF1	Insulin-like growth factor 1 ↓	[159]
IGF1R	Insulin-like growth factor I receptor ↓	[161]
BIRC5	Baculoviral IAP repeat-containing protein 5 ↓	[178]
SREBF1	Sterol regulatory element-binding transcription factor 1 ↓	[309]
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21) ↑	[117]
FOXO1A	Forkhead box O1A ↑	[145]
FOXO3A	Forkhead box O3A ↑	[146]
PTEN	Phosphatase and tensin homog ↑	[163]
PRKAB1	Protein kinase, AMP-activated, noncatalytic, β-1 ↑	[163]
TSC2	TSC complex subunit 2 ↑	[163]
DEPDC6	DEP domain-containg protein 6 (DEPTOR) ↑	[165]
TNFSF10	Tumor necrosis factor ligand superfamily, member 10↑	[175]
ATG7	Autophagy-related 7 ↑	[191]
APOB	Apolipoprotein B ↑	[266]
AQP3	Aquaporin 3 ↑	[277]
AQP1	Aquaporin 1 ↑	[285]
CASP1	Caspase 1 ↑	[294]
ALOX5	Arachidonate 5-lipoxigenate ↑	[318]
AIFM1	Apoptosis-inducing factor, mitochondria associated ↑	[325]

Table 2. Genes, whose expression is either down-regulated or up-regulated by FoxO1.

Gene symbol	Gene name	References
AR	Androgen receptor ↓	[23]
SREBF1	Sterol regulatory element-binding transcription factor 1 ↓	[24]
PPARG	Peroxisome proliferator-activated receptor gamma γ ↓	[25]
STAT3	Signal transducer and activator of transcription ↓	[26]
FSHB	FSH β-polypeptide downregulation ↓	[248]
LHB	β-subunit of LH downregulation ↓	[249]
GATA6	GATA-binding protein 6 ↑	[112,114]
FASLG	Fas ligand ↑	[237]
MTTP	Microsomal triglyceride transfer protein ↑	[267]
APOC3	Apolipoprotein C-III ↑	[268,269]

9. Conclusions and Future Perspectives

The common inflammatory skin disease acne vulgaris, a highly prevalent disease in Western countries [327,328], is related to hereditary and multiple environmental factors [329,330], especially Western style diet (hyperglycemic carbohydrates, milk and dairy products) promoting insulin/IGF-1-driven nutrigenomic aberrations [331,332] (Figure 1). The observed changes in transcriptional regulation are primarily linked to downstream IGF-1/insulin/PI3K/AKT/mTORC1 signaling and androgen/AR/mTORC2/AKT signaling, which compromise the nuclear activities of p53, FoxO1 and FoxO3. Nuclear downregulation of FoxO1 results in transactivation of AR, SREBF1, PPARγ, STAT3

and attenuation of GATA6, critical transcription factors promoting sebocyte lipogenesis (increased sebum production), inflammation and infundibular hyperkeratinization (comedogenesis), respectively. AKT-MDM2-mediated suppression of p53 modifies the expression of critical p53 downstream target genes.

In contrast, the most effective anti-acne agent, isotretinoin, attenuates all major pathogenic mechanisms of the disease. Since the introduction of isotretinoin for the treatment of cystic and conglobate acne in 1979 by Peck et al. [333], isotretinoin´s inhibitory mode of action on the pilosebaceous unit remained a miracle. In the same year p53 was detected [296].

After four decades, evidence summarized in this review allows the conclusion that isotretinoin via upregulation of p53, FoxO1 and FoxO3 counteracts all major pathways in acne pathogenesis. Isotretinoin-induced sebocyte apoptosis with sebum suppression appears to be a result of upregulation of the proapoptotic transcription factors p53 [147], FoxO1 [144] and FoxO3 [146]. However, forced p53/FoxO1/FoxO3 signaling also explains the adverse effects of isotretinoin treatment including its teratogenicity (NCC apoptosis), reduction of ovarian reserve (granulosa cell apoptosis), risk of depression (reduced hypothalamic neurogenesis), dry skin (upregulation of *AQP3*), hypertriglyceridemia (upregulation of *APOB*) and risk of intracranial hypertension (upregulation of *AQP1*) among other adverse effects.

p53-inactivation by SV40 large T antigen in immortalized sebocytes (SZ95; SEB-1) is an overlooked critical limitation of these *in vitro* sebocyte models for acne research and certain pharmacological studies that depend on adequate death signaling pathways. After a period of enthusiasm, it´s apparently time to go back to primary sebocytes and *in vivo* studies. After four decades since the introduction of isotretinoin for acne therapy, we are at the beginning to understand its mode of action on the level of transcriptomic regulation. In the future, the role of STAT3 in acne pathogenesis deserves closer investigation, as STAT3 inhibits the expression of p53 [334] and interacts with FoxO1 [26]. Another highly interesting research question should be answered: Does the upregulation of p53 by isotretinoin accelerate the process of programmed sebocyte death by holocrine secretion?

Finally, it should be realized that the reported transcriptomic deviations exhibit a significant overlap with the transcriptomic landscape observed in common cancers of Western civilization, especially prostate and breast cancer [335–337]. It is thus not surprising that epidemiological studies reported an association between severe acne during adolescence and later risk of prostate cancer [338,339] and breast cancer [340], respectively. In contrast, the transcriptional changes induced by isotretinoin are useful in the prevention and therapy of various cancers [341–345].

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Abbreviations

ACTH, adrenocorticotropic hormone
AIFM1, apoptosis-inducing factor, mitochondria associated

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AKT, Akt kinase (protein kinase B)
ALOX5, arachidonate 5-lipoxigenate
APOB, apolipoprotein B
APOCIII, apolipoprotein CIII
AQP1, aquaporin 1
AQP3, aquaporin 3
AR, androgen receptor
ARE, androgen response element
ATG7, autophagy-related 7
ATP, adenosine triphosphate
ATRA, all-trans retinoic acid
BIRC5, baculoviral IAP repeat-containing protein
BMP2, bone morphogenic protein 2
C. acnes, Cutibacterium acnes
CASP1, caspase 1
CDKN1A, cyclin-dependent kinase inhibitor 1A (p21)
ChP, choroid plexus
CRABP2, cellular retinoic acid binding protein 2
DEPTOR, DEP-domain containing mTOR-interacting protein
EFTUD2, elongation factor Tu GTP-binding domain-containing 2
FABP5, fatty acid binding protein 5
FADS2, fatty acid desaturase 2
FASLG, Fas ligand
FGFR2, fibroblast growth factor receptor 2
FLG, filaggrin
FoxO1, forkhead box O1
FoxO3, forkheadbox O3
FSH, follicle stimulating hormone
FSHB, FSH β-polypeptide downregulation
FST, follistatin
GAP, GTPase-activating protein
GATA6, GATA binding protein 6
GC, granulosa cell
GH, growth hormone
HIF-1α, hypoxia-inducible factor 1α
HIF-2α, hypoxia-inducible factor 2α
IGF-1, insulin-like growth factor 1
IGF1R, insulin-like growth factor receptor
IL-1β, interleukin-1β
IL-6, interleukin 6
IL-8, interleukin 8
IL-17, interleukin 17
INSR, insulin receptor
Isotretinoin, 13-cis retinoic acid
LEP, leptin
LH, luteinizing hormone
LHB, β-subunit of LH downregulation
MDM2, MDM2 protooncogene
mTORC1, mechanistic target of rapamycin complex 1
mTORC2, mechanistic target of rapamycin complex 2
MTTP, microsomal triglyceride transfer protein
NC, neural crest
NCC, neural crest cell
OVOL1, ovo-like 1
PGN, peptidoglycan
PI3K, phosphoinositide 3-kinase
PLIN2, perilipin 2
POMC, proopiomelanocortin

PRKAB1, protein kinase, AMP-activated, noncatalytic, β-1
PPARβ/δ, peroxisome proliferator-activated receptor β/δ
PPARγ, peroxisome proliferator-activated receptor γ
PTEN, phosphatase and tensin homolog
RAI1, retinoic acid-inducible gene 1
RAPTOR, regulatory associated protein of mTOR;
RAR, retinoic acid receptor
RARE, retinoic acid response element
RICTOR, rapamycin-insensitive companion of mTOR
RHEB, Ras protein homolog enriched in brain
RORγt, retinoic acid receptor-related orphan receptor-γt
SG, sebaceous gland
SMAD2, SMAD family member 2
SREBF1, sterol regulatory element binding transcription factor 1
STAT3, signal transducer and activator of transcription 3
SV, Simian virus
TCOF1, treacle ribosome biogenesis factor 1
TGFβ, transforming growth factor beta
TGFB2, transforming growth factor beta 2
TLR2, toll-like receptor 2
TLR4, toll-like receptor 4
TNF-α, tumor necrosis factor-α
TNFSF10, tumor necrosis factor ligand superfamily, member 10
TP53, tumor protein
TRAIL, tumor necrosis factor-related apoptosis inducing ligand (TNFSF10)
TSC1, TSC complex subunit 1 (hamartin)
TSC2, TSC complex subunit 2 (tuberin)
TSH, thyroid stimulating hormone
VLDL, very low density lipoprotein

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