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SHORT COMMUNICATION

Upregulation of PI3K/AKT/mTOR, FABP5 and PPARβ/δ in Human Psoriasis and Imiquimod-induced Murine Psoriasiform Dermatitis Model

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Psoriasis is a common, and currently incurable chronic immune-mediated skin disease (1), with incompletely understood etiology partially due to the unavailability of animal models that can emulate major features of the disease. The phospho-inositol-3 kinase (PI3K)/protein kinase B(Akt) and mammalian target of rapamycin (mTOR) signaling which regulate metabolism, cell proliferation, survival, apoptosis, and frequently deregulated in diverse cancers (2, 3), has recently emerged as a clinically relevant target for inflammatory diseases including psoriasis (4, 5). This pathway is tightly regulated through feedback loops in part via 2 mTOR complexes, (C1 and 2), with linkage through Akt. PI3K activation triggers the phosphorylation of a 3-hydroxyl group, which then activates Akt kinase and promotes keratinocyte hyperproliferation and inhibit differentiation, as observed in psoriasis (4). Initial clinical data suggest that second-generation inhibitors (targeting both mTOR and PI3-K kinases) provide therapeutic benefit for psoriasis (6). Additionally, psoriasis-associated epidermal-type fatty acid-binding protein (FABP-5) involved in cytosolic fatty acid-transport, as well as the peroxisome proliferator-activated receptor β/δ (PPAR β/δ), a ligand-activated transcription factor and member of the nuclear hormone receptors superfamily, are upregulated in psoriasis (7–9). The goal of this study was to assess whether mTOR, its upstream (PI3K and Akt) and downstream S6K1 (specifically S6Ser235/236 and S6Ser240/244) targets, as well as FABP5 and PPARβ/δ, are overexpressed in inflamed toll-like receptor-7/8 ligand imiquimod (IMQ)-induced Balb/c mouse skin lesions and, whether their expressions simulate those observed in inflamed skin lesions from untreated psoriatic patients compared with matched controls.

MATERIALS AND METHODS

Male Balb/c mice, 6–8-week-old (Harlan laboratories, Madison, WI) were used in this study. Mice received a daily topical dose of 62.5 mg of commercially available IMQ cream (5%) (Aldara, 3M Pharmaceuticals), on the shaved back for 5 days, and received the same daily booster dose for 9 additional days for a total of 14 consecutive days to achieve optimal chronic inflammation. IMQ-treated ($n = 6$) and pair-matched control ($n = 6$) mouse skin tissues were freshly harvested as earlier described (10), for details of histological analyses see Appendix S1[1]. For human samples, different clinical grades lesional skin from untreated patients with active psoriasis ($n = 12$), fresh healthy skin (n=10) taken after written and informed consent, were processed for histology and immunostaining (see Appendix S1[1]). Images were generated using the Nuance® EX/FX and Vectra™ (PerkinElmer, Boston, MA) multiplexing image technology platform and analyzed by the inForm software. We also analyzed changes in protein expression by western blotting using antibodies outlined in Appendix S1[1].

RESULTS AND DISCUSSION

Consistent with previous reports (4, 9, 11), we observed upregulation of the expression and activation of PI3K/Akt and mTOR kinases, and PPARβ/δ and FABP5 in human psoriatic skin lesions compared to matched control skin. Importantly, we provide evidence that these kinases and markers are also overexpressed in inflamed skin lesions of IMQ-induced mouse psoriasis-like skin model compared to matched controls. PI3K phosphorylation activates Akt which regulate multiple targets and cellular processes (2, 12). We observed overexpression of PI3K in inflamed human psoriatic and IMQ-induced Balb/c mouse skin lesions (Fig. S1[1]). Full activation of Akt requires dual phosphorylation on Thr^{308} by the activation loop via the PI3K/ PDK1 and on Ser^{473} by mTORC2 which also promotes Thr^{308} phosphorylation by PDK1 (13). Akt hyperphosphorylation was observed at two sites; Ser^{473} (strong staining in the suprabasal/differentiating layers with occasional punctate staining in the basal layers), and Thr^{308} (entire epidermis including part of the dermis) in human psoriatic (Fig. S2 A[a, b][1] and in IMQ-induced (Fig. S2B[aa–ab][1]) skin lesions, compared with respective controls (Fig. S2A[g, h] and B[ag–ah][1]). The serine/threonine protein kinase mTOR interacts with several proteins within the rapamycin-sensitive mTORC1 and the rapamycin-resistant mTORC2 pathways. Both complexes are differentially regulated and can be activated by autophosphorylation at Ser^{2481} and by Akt-mediated phosphorylation at Ser^{2448} (3, 14). Here, mTOR was hyperphosphorylated both on Ser^{2481} (illustrative of a predominant mTORC2 activation) and to some extent on Ser^{2448} (indicative of mTORC1 activation) in human psoriatic (Fig. S2A[c, d][1]) and IMQ-induced (Fig. S2B[ac–ad][1]) skin lesions

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(14). Interestingly, IMQ-treated lesions prominently expressed both phospho-Akt(Ser^{473};Thr^{308}) and phospho-mTOR(Ser$^{2481;\ 2488}$) in the entire epidermis/upper dermis, mimicking severe psoriasis (Fig. S2B [aa–ad] and [ag–aj][1]) compared to controls (Fig. S2A[i, j][1] and B(aI–aJ)[1]) showing weak expression.

Analyses with pan- and phospho-specific antibodies showed that IMQ-induced activation of Akt(Ser^{473}) and downstream targets (p44/42, S6 and p90RSK, and Stat3 in IMQ-treated skin lysates and sections (Figs S1 and S3A[1]). Activated mTORC1 transmits signal by activating ribosomal protein S6 kinase-1(S6K1), and 4E-BP1 which regulate eukaryotic mRNA translation initiation and protein synthesis, whereas mTORC2 regulates proliferation, growth and cytoskeleton remodeling via Akt activation. S6 activity is a widely accepted measure of mTOR activity and recently reported to be activated in differentiating layers of inflamed psoriatic skin (15). Human psoriatic skin exhibited strong S6 phosphorylation at (Ser$^{235/236}$ and Ser$^{240/244}$) predominantly in the suprabasal/differentiating epidermal layers, whereas basal layers showed only a few cell with punctate staining (Fig. S2A[e, f][1]). Consistent with human psoriasis, prominent overexpression of both phospho-S6 isoforms was observed in the entire epidermis including part of the dermis in IMQ-treated mouse skin tissues (Fig. S2B[ae–af][1]). However, differential phospho-S6 expression pattern was observed among patients with mild, moderate/severe phenotypes. Control human and mouse skins revealed basaline phospho-S6 expression in stratum granulosum (Fig. S2A[k, l][1] and B[ak–al][1]). By immunofluorescence with pan-lymphocytic marker (CD45) we observed the association of S6 phosphorylation with inflammation being more extensive in severely inflamed lesions than in less inflamed areas both in human psoriatic (Fig. S2A[f][1]) and IMQ-induced (Fig. S2B[af][1]) lesions. Intriguingly, while PI3K is overexpressed in the basal layers, Akt/mTOR and S6 are hyperactivated throughout suprabasal/differentiating epidermal layers in lesional psoriatic skin, IMQ-induced lesions in mice shows hyperactivation of these makers in the entire epidermis, recapitulating severe psoriasis.

FABP-5 expression was significantly increased in inflamed psoriatic lesions (Fig. S3B[1] top panel and inset). We also report for the first time that IMQ-induced skin lesions recapitulate similar FABP5 staining (Fig. S3C[1] and inset). We observed that PPAR β/δ, known to be associated with disease-promoting role in psoriasis (11) showed strongly increased cytoplasmic and nuclear activation both in inflamed human psoriatic and IMQ-induced lesions compared with only cytoplasmic basal cell staining in controls (Fig. S1[1] and Fig. S3E[1], inset). Interestingly, in IMQ-induced lesions prominently overexpressed PPARβ/δ was particularly observed in suprabasal/differentiating compartments, the hair follicles and fibroblastic dermal cells (Fig. S3E[1] and insets).

Our data reveal that topical application of IMQ is sufficient to activate PI3K/Akt and mTOR kinases, as well as the upregulation PPARβ/δ and FABP5 in the murine skin, in addition to recapitulating other reported pathological features characteristic of psoriasis. Our study also concurs with previous data (4, 9, 11), that activation of PI3K/Akt/mTOR signaling is involved in the pathogenesis of psoriasis. Therefore, we conclude that the IMQ-induced murine psoriasis-like inflammation is a useful model that can be tweaked for preclinical evaluation and development of pharmacologic agents targeting the PI3K/Akt/mTOR pathway and other disease markers for the management of psoriasis.

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The authors declare no conflict of interest.

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