Activation of Toll-like Receptors 1, 2, 4, 5, and 7 on Human Melanocytes Modulate Pigmentation

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Human melanocytes are not merely pigment-producing cells. They are also capable of phagocytic functions, contributing to inflammatory responses. Indeed, there is evidence that human melanocytes and melanin inhibit the proliferation of bacterial, fungal, and parasitic infections in the epidermis and dermis. For example, radicals and other compounds produced during melanogenesis are believed to exert strong antimicrobial activity.

Toll-like receptors (TLRs) are part of the innate immune system involved in the response to microbial infections. There are at least 10 members of the TLR family identified in mammals. TLRs recognize a wide range of microbial ligands, including lipopolysaccharides (LPS), bacterial lipoproteins, bacterial heat shock proteins, viral single- and double-stranded RNA, and bacterial flagellin. The innate immune system governs the interconnecting pathways of microbial recognition, inflammation, microbial clearance, and cell death. The pigmentary system may produce more melanin, or suppress melanization, in response to inflammatory events. It is therefore possible that TLR activation in melanocytes may play a role in pigmentation modulation.

The objective of this study was to investigate whether normal human melanocytes express all TLR members, and analyze pigmentation changes upon TLR stimulation. To investigate whether TLRs 1–10 are constitutively expressed in cultured normal human melanocytes, we performed RT-PCR, immunocytochemistry and Western blot analysis. The primers sequences used were as follows: TLR1 sense oligonucleotide, 5’-AAAAAGAAGACCCCTGAGGGCC-3’ and anti-sense oligonucleotide, 5’-TCTGAAGTCAGCTGACCCTTGAGGGCC-3’; TLR2 sense oligonucleotide, 5’-AAC
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Fig. 1. Toll-like receptors (TLRs) are expressed in human melanocytes. (A) Expression of TLR1-10 was assessed by RT-PCR. The protein expression was detected by immunocytochemistry (B: ×200) and Western blotting (C). Negative controls were made by applying normal rabbit serum before treatment with primary antibody.
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Effects of toll-like receptor (TLR) ligands on pigmentation. Melanocytes were treated with Pam3CSK4, PolyIC, LPS, flagellin or imiquimod, a ligand (1 ∼ 10 μg/ml) of TLR1/2, 3, 4, 5, 7, 9, and 10 for 5 days. (A) Melanin content was determined by measuring the absorbance at 490 nm. The values indicate the mean±SD of three independent experiments. *p < 0.05. (B) Zebrafish was treated with ligands (20 or 40 μg/ml) for 24 hrs and was visualized under microscope. The results were reproducible in three independent experiments. Magnified view (Inset).

Treatment of 10 μg/ml flagellin or imiquimod reduced melanin contents to 91 or 85% (mean±SD of control, n = 3, p < 0.05) of the control cells (100%), respectively. There was no change with PolyIC treatment. The effects of TLR ligands was further investigated using the zebrafish model system. As shown in Fig. 2B, flagellin (20 μg/ml) and imiquimod (40 μg/ml) inhibit zebrafish body pigmentation. However, there was no remarkable change with treatment of Pam3CSK4 or LPS (data not shown). Taken together, these results suggest that activation of TLR2, 4, 5 and 7 modulate melanocyte pigmentation.

The present study demonstrated the in vitro expression of a panel of TLRs in normal human melanocytes. Bacterial cell wall components are recognized by TLR1, 2, 3, 5, and 6, known as extracellular TLRs. We showed that treatment of TLRs (1/2, 4 and 5) with synthetic bacterial lipoprotein, PolyIC, LPS and bacterial flagellin modulated melanocyte pigmentation. TLR3, 7, 8, and 9 are located in the cytoplasm and recognize mainly viral components. In our study, stimulation with imiquimod and synthetic TLR7/8 ligand reduced melanocyte pigmentation. It was shown that flagellin and imiquimod treatment also reduced zebrafish body pigmentation. It appears that zebrafish body pigmentation was too intense to show the subtle changes of increased pigmentation. Taken together, these results suggest that TLRs in melanocytes may play a role in microbial- or inflammation-related pigmentary changes. However, further investigations are needed to confirm the functional role of TLRs in pigmentation and validate the outcomes of this study, since it remains unclear whether pigmentary changes by TLRs ligands in this study were related to TLR activation.

In summary, we have demonstrated TLRs expression in normal human melanocytes. Further investigations are warranted to determine whether melanocyte response to TLR ligands may play a role in skin pigmentary changes.

REFERENCES
