厚生労働科学研究費補助金（免疫アレルギー疾患予防・治療研究事業）
分担研究報告書

浮遊培養法を用いた表皮角化細胞の重層化に伴う分化に関する研究

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研究要旨
表皮角化細胞は基底膜から遊離することにより分化を開始する。Poly-HEMA コートしたティッシュを用いた浮遊培養て、各種分化マーカーか発現したことにより、浮遊培養て角化細胞の分化か誘導てきることか明らかとなった。基底膜より遊離することにより重層化する表皮角化細胞は、基底膜への接着により制御されている PI3 kinase を介して分化を制御していると考えられる。

A 研究目的

表皮角化細胞は基底膜から遊離することにより分化を開始し、多層構造をもつ表皮を形成する。分化機構は、構造的な分化を制御すると同時に自然免疫をも制御していると考えられるか、表皮角化細胞の重層化に伴う分化制御機構は明らかではない。しかし、通常の培養方法では、単層の角化細胞しか培養することかてきす、基底膜との接着による分化制御機能の解析は困難であった。そこて、この研究では表皮角化細胞を分化させる浮遊培養法の確立し、重層化に伴う分化制御機構を明らかにすることを目的とする。

B 研究方法

1) 角化細胞の浮遊培養

Polyhydroxyethyl methacrylate (poly-HEMA) を用いて浮遊培養法を確立した。Poly-HEMA を 10mg/ml てユタノールに溶解し、4ml を 6 cm ティッシュに添加し、クリーンベンチ内て乾燥させる。さらにもう一度同様に 4 ml の poly-HEMA 溶液を加え乾燥させた後、PBS て洗浄する。

正常ヒト角化細胞は無血清培地である MCDB153 を用い、100 mm の type 1 コラーゲンコートのシャーレて培養する。細胞密度か 70~80 %程度になったら 0 25 %トリプシン，0 05 % EDTA 混合用液を用い、細胞の回収を行い、上記の poly-HEMA ティッシュに播種密度 200 万/ティッシュて播種する。細胞の回収は PBS て行う。

2) 分化マーカー発現の検討

浮遊培養の後、細胞を回収し mRNA を抽出する。Rnonuclease protection assay (RPA)法にて、transglutaminase-1, involucrin loricrin, keratin-1 -10 などの分化マーカーの発現を検討する。さらに、Western blot 法にて involucrin keratin-1, -10 タンパクの発現を検討する。

3) Phosphatidylinositol (PI)-3 kinase 活性測定

細胞は回収後 lysis buffer (20 mM tris-HCL, pH7.5, 1mM MgCl2 1mM CaCl2 0 2M NaCl 10% glycerool 1% NP-40)て抽出し、抗 p110 抗体にて免疫沈降する。免疫複合体を kinase buffer て浮遊した後、[γ ^{32}P]ATP と 37 C、20 分インキュベートする。反応終了後、脂質を薄層クロマトクラフィーにて分離する。

C 結果

Poly-HEMA てコートしたプレートを用い

て培養したところ、角化細胞はほとんど付着せず、浮遊状態で培養できることが明らかとなった。浮遊培養後、分化マーカーの発現を RPA 法にて検討したところ、transglutaminase-1, involucrin, loricrin, keratin-1 -10 の mRNA 発現が増強しており、さらにタンパクレベルでも involucrin keratin-1, -10 の発現が増加していた。この分化マーカーは BHE を培地に添加しない条件で発現が増強していた。ついで、PI3 kinase の活性を測定した。その結果、単層培養の条件では、PI3 kinase の活性が認められたが、浮遊培養を行うと、PI3 kinase の活性は著しく低下した。

D 考察

Poly-HEMA コートしたティッシュを用いた浮遊培養で、各種分化マーカーが発現したことにより、浮遊培養で角化細胞の分化が誘導できることが明らかとなった。また、PI3 kinase の活性は poly-HEMA コートのティッシュを用いた培養で著しく低下したことから、PI3 kinase の活性は細胞外マトリックスへの接着により制御されており、PI3 kinase の活性が分化を制御している可能性がある。

E 結論

基底膜より遊離することにより重層化する表皮角化細胞は、基底膜への接着により制御されている PI3 kinase を介して分化を制御していると考えられる。従って、PI3 kinase も自然免疫の細胞内制御因子である可能性がある。

F 健康危険情報

なし

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