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EFFECT OF RETINOIC ACID ON cAMP DEPENDENT PROTEIN PHOSPHORYLATION IN PSORIATIC FIBROBLASTS

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Retinoic acid treatment of psoriatic fibroblasts increases the activity of cyclic AMP dependent protein kinase. In this study we report that retinoic acid treatment of cultured psoriatic fibroblasts modifies their subsequent cAMP dependent protein phosphorylation. In the soluble fraction of normal fibroblasts cAMP clearly enhances the in vitro phosphorylation of proteins of MW 37,49,54,56,68,83 kD while retinoic acid treatment of the same cells results in a decrease of the cAMP dependent phosphorylation of the first five of the same proteins. In contrast, in psoriatic fibroblasts from psoriatic patients retinoic acid either has no effect or increases the cAMP dependent phosphorylation of some of these proteins. Moreover the phosphorylation of a protein of MW 54 kD, undetectable in untreated psoriatic cells, is more phosphorylated in the presence of cAMP after retinoic acid treatment. The appearance of this phosphorylated proteins is time dependent and dose dependent upon the addition of retinoic acid . These in vitro phosphorylation results suggest that retinoic acid treatment of psoriatic fibroblasts change the level of cAMP dependent phosphorylation of some cytosolic proteins. These specific phosphorylations could be implicated in a variation of cell functions.

Retinoids, derivatives of vitamin A, play an essential role in regulation of growth of many different cell types (1-3) and are required for normal epithelial cell differentiation (4).

Retinoic acid derivatives have been used with success as therapy in psoriasis. These compounds have been shown to increase cAMP dependent protein kinase activity during differentiation of tumoral cells (5,6) and to change the protein phosphorylation pattern in these cells (7).

We recently showed in psoriatic fibroblasts a deficiency of cAMP dependent protein kinase and reported that this deficiency in psoriatic erythrocytes was correlated with the severity of the disease (8).

ABBREVIATIONS RA: Retinoic Acid
cAMP: cyclic Adenosine Monophosphate

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In vitro treatment of psoriatic fibroblasts with retinoic acid induces an increase either in cAMP dependent protein kinase activity or in the photoaffinity labelling with 8-Azido[^{32}P]cAMP of both regulatory subunits RI and RII of these enzymes (9). For this reason we were interested in the in vitro cAMP dependent phosphorylation of proteins from normal and psoriatic fibroblasts before and after retinoid acid (RA) treatment.

MATERIALS AND METHODS

Trypsin (2X crystallised, collagenase (type II-5), cAMP, and kemptide were purchased from Sigma (St. Louis, Missouri). Ham's F12 medium and glutamine were from Flow Laboratories (Puteaux, France) and fetal calf serum from Seromed (Berlin, F.R.G.). All-trans-vitamin A (retinoic acid), was a generous gift from Hoffman-LaRoche (Basel-Swizerland). [γ^{32}P]ATP (30 Ci/mmol) was obtained from Amersham (Les Ulis, France), and [8-Azido^{32}P]cAMP (20 Ci/mmol) from ICN Pharmaceuticals (Cleveland, Ohio). Low and high molecular weight standarts were from Biorad (Richemond, California).

Fibroblast cultures were established as previously described (8). The cells were used after their fourth and fifth passage. Where indicated RA dissolved in absolute ethanol was added one day after plating to give a final concentration of 1μM. The final concentration of ethanol in the medium did not alter cell growth, morphology or in vitro protein phosphorylation.

Preparation of cells for in vitro protein phosphorylation: Control and RA treated cells were harvested at various times following the RA addition. Growth medium was aspirated from the dishes and the cells washed three times with ice cold buffer (50 mM MES, 10 mM $MgCl_2$, 0.25 M sucrose pH 6.2) and scraped into this buffer with the addition of leupeptin (20μg/ml). The cells were then homogeneized in a Dounce homogenizer (27 strokes) and the homogenate centrifuged at 12 000 g for 15 minutes. Aliquots of both supernatant and particulate fractions from all samples were assayed for protein content using a fluorometric assay with bovine serum albumin as standard(10).

Protein phosphorylation and gel electrophoresis: Supernatant or particulate fractions (50 μg of protein) were autophosphorylated in a total volume of 100 μl containing 50 mM MES, pH 6.2, 10 mM Mg Cl_2 , 5.5 μM [γ^{32}P] ATP, 10μM cAMP and 1mM 3 isobutyl-1-methylxanthine for 3 minutes. The reaction was stopped by the addition of 20 μl of SDS sample buffer (9% SDS, 15% glycerol, 3 mM EDTA, 30 mM Tris buffer pH 8). Samples were treated at 100°C for 2 minutes and 2 μl of 2 mercaptoethanol and 5 μl of 0.1% bromophenol blue in 50% glycerol were then added. Samples were analyzed by SDS-polyacrylamide gel electrophoresis using a 6% to 15% gradient separating gel and 2% stacking gel. Gels were stained for proteins with Coomassie blue, destained and dried and exposed against Amersham MP films for one to three days at -80°C. Densitometric scannig of the autoradiograms was performed using a Beckmann densitometer.

RESULTS

We showed in previous studies that RA treatment of psoriatic fibroblasts (uninvolved and involved skin) in culture induced an increase in cAMP dependent protein kinase activity (9) and also an increase of the amount of 8-Azido[^{32}P]cAMP bound to the soluble regulatory subunits RI and RII (9). In contrast either no change or a decrease in cAMP dependent protein kinase activity occured upon RA treatment of normal fibroblasts. These results led us to investigate

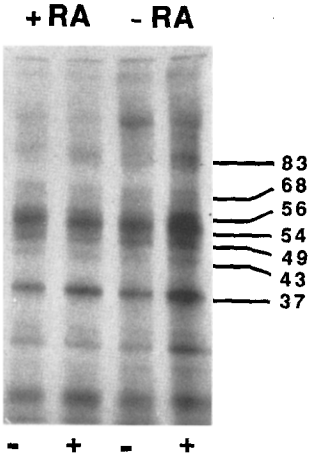

Figure 1: The effect of RA treatment of normal fibroblasts on subsequent in vitro phosphorylation. Normal fibroblasts were treated or not for 48 h with 1μM RA. Cells were then harvested and in vitro phosphorylation performed with 12 000 g supernatant in presence (+) or absence (-) of 1μM cAMP.

whether some cellular proteins were phosphorylated in a cAMP dependent manner in the presence or absence of RA.

In our experimental conditions we could not detect any change in phosphorylation in the presence of cAMP in the particulate fractions either in normal or in psoriatic cells and no modification after RA treatment.

In the 12 000 g supernatant of normal fibroblasts seven bands were phosphorylated more intensively in the presence of cAMP, three prominent bands of MW respectively 37, 56, 83 kD, and four weaker bands of MW 43,49,54,68 kD (Fig 1).When normal fibroblasts were treated with RA cAMP decreased the subsequent in vitro phosphorylation of four bands (37,43,49,56 kD) or slighty modified the phosphorylation of three bands (54,68,83 kD).(Fig 1 and 2).

In psoriatic fibroblasts, only five bands (37,43,56,68,83 kD) are phosphorylated in the presence of cAMP. In contrast to normal cells the RA treatment of all type of psoriatic fibroblasts induced the phosphorylation of protein at 54 kD and in one patient at 49 kD (Fig 2 and 3).

We previously showed that the changes of cAMP dependent protein kinases in psoriatic fibroblasts were dependent upon the concentration of RA used in treating the cells (9). We therefore studied whether the changes in protein phosphorylation were also dependent upon RA concentration. Psoriatic fibroblasts were treated for 2 days with either 0.01, 0.1, 1μM RA. The

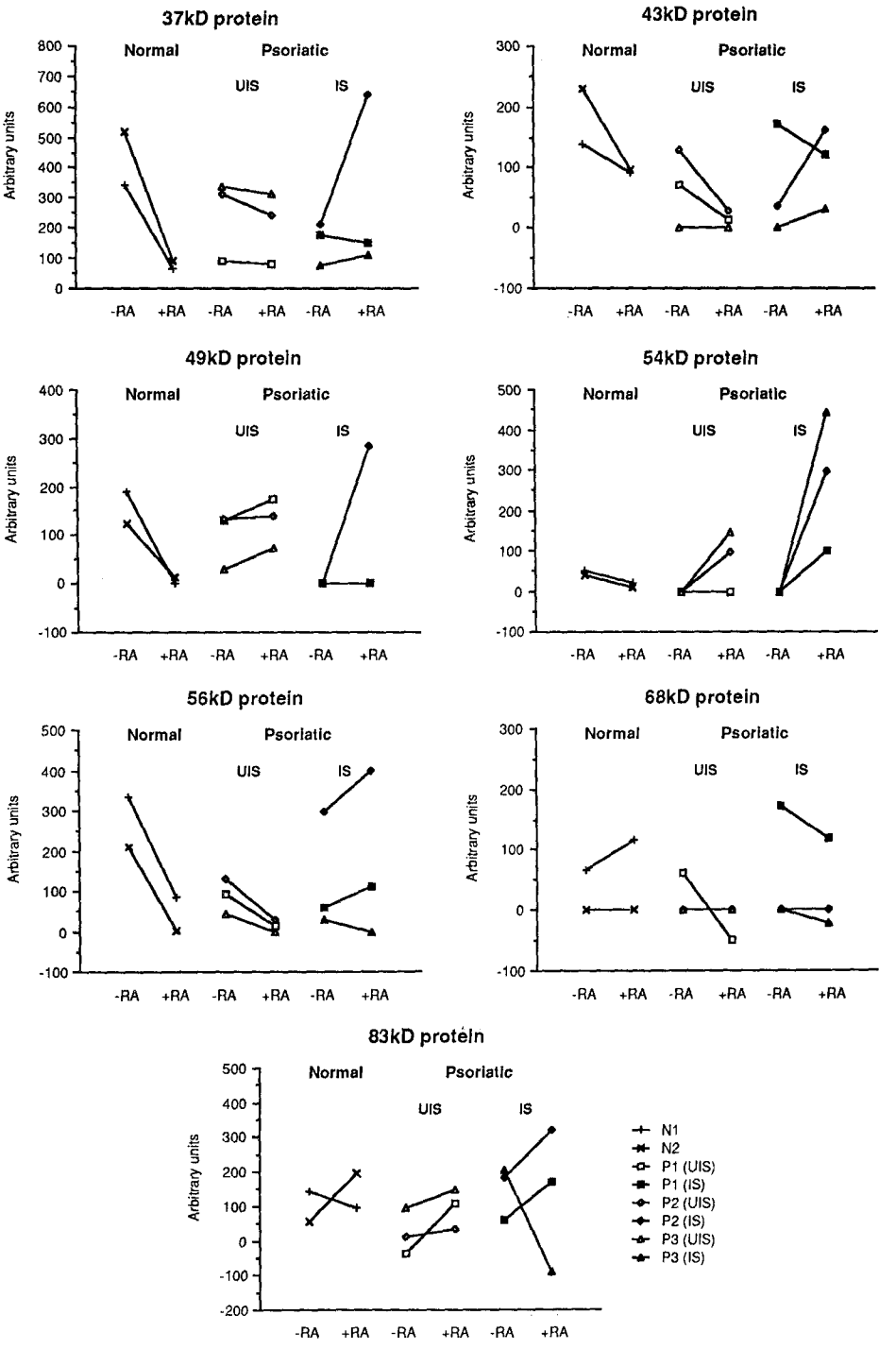

Figure 2: cAMP dependent phosphorylation of soluble proteins of normal and psoriatic fibroblasts in presence (+RA) and absence (-RA) of RA treatment after SDS PAGE. An arbitrary quantitation of the cAMP dependent phosphorylation of the seven protein bands which is calculated from the scanner integration of the intensity of the autoradiograph. The value represents the amount of P^{32} incorporated in the protein band in presence of cAMP minus the amount in absence of cAMP. N = Normal(2 subjects), P = Psoriatic (3 patients).
UIS = UnInvolved Skin, IS = Skin

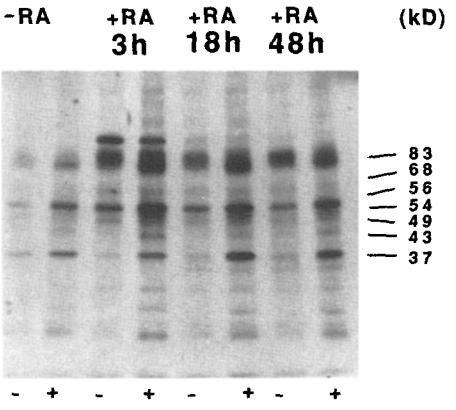

Figure 3: Time couse of the effect of RA treatment of psoriatic fibroblasts on subsequent in vitro cAMP dependent phosphorylation. Cells were treated with 1μM RA for 3, 18 or 48 hours in involved fibroblasts in presence (+) or in absence (-) of 1μM cAMP during in vitro phosphorylation.

phosphorylation changes were maximal at a RA concentration of 1μM and did not occur at a concentration of 0.01μM (data not shown). In addition studies on the time course of RA action (1μM RA) on the pattern of the cAMP dependent protein kinases revealed that the increase was observed as early as three hours after RA addition to the cell culture medium. Similarly we explored whether alterations in in vitro protein phosphorylation were dependent upon the duration of RA treatment (Fig 2). The 54 kD phosphorylated protein appeared in the soluble fractions of psoriatic cells after three hours of RA treatment and was not further increased with longer periods of RA treatment. In some cases, (for example the 54 kD proteins and 49 kD), similar changes in phosphorylation were observed in uninvolved and involved skin fibroblasts of psoriatic patients. However since the pattern of change was not consistent amongst all the proteins it is not possible to draw further conclusions concerning effects in the two situations.

DISCUSSION

This study demonstrates that RA treatment of human fibroblasts can alter their subsequent in vitro cAMP dependent phosphorylation.

RA induced the phosphorylation of a protein band of MW 54 kD in the three psoriatic patients studied, which was enhanced by cAMP. The effect of RA on the phosphorylation of the 54 kD protein was time and dose dependent. It will be of interest to indentify further the 54 kD protein(s) and to assess its precise role in psoriatic fibroblasts.

Although these results do not establish definitively that the increase in cAMP dependent phosphorylation in retinoic acid treated psoriatic cells is due to increased activity of cAMP dependent protein kinases, it is interesting to note the similarity in time course and dose dependency of the action of RA on cAMP dependent protein kinase activities and on subsequent in vitro cAMP dependent phosphorylation of cytosolic proteins.

Among the numerous biochemical effects of RA on cells in culture (10), the changes in cAMP dependent phosphorylation observed in psoriatic cells is one of the earliest. As soon as three hours after the initiation of RA treatment, changes in phosphorylation of cytosolic proteins were observed. These effects may have a critical role in regulating psoriatic fibroblast functions which in turn have a preeminent role in the appearance of psoriatic lesions (11).

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