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Epidermal Growth Factor Receptor Expression Related to Differentiation Capacity in Normal and Transformed Keratinocytes

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Epidermal growth factor (EGF) and Ca^{2+} have been indicated to play a major role in skin development. We have used normal keratinocytes, SV40-transformed keratinocytes (SVK_{14}) and various squamous carcinoma cell (SCC) lines as in vitro model system to study the effect of the extracellular Ca^{2+} concentration on EGF-receptor expression in relation to the capability of cells to differentiate. The cell lines used exhibit a decreasing capacity to differentiate in the order of keratinocytes ~SVK_{14} > SCC-12F2 > SCC-15 > SCC-12B2 > SCC-4, as judged from Ca^{2+}-ionophore-induced cornified envelope formation.

Under normal Ca^{2+} conditions, all cell lines (except for SCC-15) exhibited two classes of EGF-binding sites. The number of low-affinity binding sites increased considerably as cells were less able to differentiate, while the apparent dissociation constant (k_d) was similar in all cell lines. In contrast, the properties of high-affinity EGF binding varied in the various cell lines without a clear relationship to the degree of differentiation capacity.

Lowering the extracellular Ca^{2+} concentration to 0.06 mM resulted in a decrease of Ca^{2+}ionophore-induced cornified envelope formation, demonstrating the decreased ability to differentiate under these conditions. The decreased ability to differentiate was accompanied by a marked increase in the number of EGF-binding sites, but without a change of the k_d. Furthermore, no high-affinity EGF-binding sites were detectable under these conditions. Finally, addition of Ca^{2+} to low Ca^{2+}-cultured cells caused a rapid decrease of EGF binding in all cell lines, most prominently in normal keratinocytes and SCC-12F2 cells.

The data presented demonstrate: (*a*) The combination of normal keratinocytes, SVK_{14} and the various SCC lines provides an attractive model system to study differentiation in vitro; (*b*) EGF-receptor expression is related to the state of differentiation, both phenomena being sensitive to the external Ca^{2+} concentration; and (*c*) EGF-receptor expression is related to the capability of cells to differentiate.

Earlier studies have indicated the skin of a variety of species, including man, as a major target for epidermal growth factor (EGF). Thus it has been shown that EGF caused thickening of neonatal epidermis in vivo [1] and in organ culture [2], while intraperitoneally injected EGF concentrated specifically in the epidermis [3]. In expanding cultures, in which basal epidermal cells were able either to multiply or to differentiate, EGF reduced the proportion of cells possessing cornified envelopes (i.e., differentiating cells) [4], while EGF was able to increase

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the culture life of keratinocytes in vitro [5] suggesting strongly that EGF arrests the cells in a proliferative state. In addition, it was shown that cultured keratinocytes lose their ability to bind EGF during differentiation in vitro [6, 7]. These results suggest that EGF plays a central role in the development of the epidermis.

An equally important factor in skin development, as compared to EGF, appears to be Ca^{2+}. Lowering the extracellular Ca^{2+} concentration to 0.05–0.1 mM caused a high proliferation activity in cultured mouse epidermal cells, while addition of Ca^{2+} to these cultures resulted in induction of differentiation [8–10]. Furthermore, the Ca^{2+}-induced terminal differentiation was accompanied by a decrease in the EGF-binding capacity [11]. The role of Ca^{2+} in the regulation of keratinocyte proliferation and differentiation might occur in vivo as well, as shown by the elegant studies of Malmqvist et al. [12]. Using proton and electron microprobe analysis [12, 13] these authors demonstrated large variations in Ca^{2+} content in the skin, the basal layer (proliferating cells) contained an extremely low Ca^{2+} content, while the upper layers (differentiating and differentiated cells) contained a 20-fold increased Ca^{2+} content. These observations strongly point to EGF and Ca^{2+} as major modulators in keratinocyte proliferation and differentiation. However, the study of the mechanism of action of EGF and Ca^{2+} in skin development is highly complicated due to the complex nature of the skin. In this respect, an attractive model system has been obtained by the development of a number of squamous carcinoma cell (SCC) lines, exhibiting a decreasing capacity to differentiate [14]. We have used these cell lines in addition to normal keratinocytes and SV40-transformed keratinocytes (SVK_{14}) to study the effect of the external Ca^{2+} concentration on EGF-receptor expression as related to the differentiation capacity of the cells.

In this paper we demonstrate that in keratinocytes EGF receptor expression is strongly related to their ability to differentiate. Whereas the number of EGF receptors observed in normal keratinocytes was comparable to that seen in other cell types [15–17], this number was significantly higher in transformed squamous carcinoma cell lines. Furthermore, lowering the extracellular Ca^{2+} concentration resulted in a marked decrease of the ability to differentiate in normal keratinocytes and SVK_{14} cells and to a lesser extent in the SCC lines, these changes being accompanied by a 2- to 5-fold increase in the number of EGF receptors per cell. The mechanism underlying the regulation of EGF-receptor expression will be discussed in relation to recently obtained results regarding low density lipoprotein (LDL) receptor expression and cholesterol synthesis in the same cell lines under similar conditions [18–20].

MATERIALS AND METHODS

Cell Lines and Culture Conditions

Juvenile human epidermal keratinocytes were cultured using Rheinwald-Green feeder layer technique [14]. The SV40-transformed keratinocytes (SVK_{14}) were kindly provided by Dr J. Taylor-

Papadimitriou [21] and human squamous carcinoma cell (SCC) lines by Dr J. Rheinwald [14]. The cells were cultured in a mixture of Dulbecco-Vogt and Ham's F12 medium (3 : 1) supplemented with 5% fetal calf serum (FCS), 0.4 μg hydrocortisone/ml, 10^{-6} M isoproterenol (normal and SV40-transformed keratinocytes) and 10 ng epidermal growth factor (EGF)/ml (normal keratinocytes) [20]. For EGF-binding studies, normal keratinocytes were replaced on EGF-free medium after reaching confluency at least 3 days before the experiment. This treatment caused no severe effects on the cells, both on morphological and biochemical level. For the low-calcium cultures calcium-free Dulbecco-Vogt medium mixed with standard Ham's F12 medium (3 : 1) and supplemented with 5% chelex-treated FCS [22] was used. The final calcium concentration was 0.06 mM, as determined by flame photometry.

Cornified Envelope Formation

The capacity of cornified envelope formation was determined according to Rice & Green [23], as described in detail previously [18].

Cell Surface Area Measurement

The cell surface area was measured as described [24]. Trypsinized cells were suspended in medium made progressively hypotonic by adding H_2O. The maximum diameter attained by the cells was measured from photographs. The mean cell surface area was then estimated assuming the maximally swollen cell to be spherical. This method has been shown previously to provide reliable results [24].

EGF Binding

[^{125}I]EGF binding was measured on monolayer cultures in DMEM (pH 7.4) containing 0.1% bovine serum albumin, buffered with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES) as described previously [15]. Scatchard analysis was performed using the LIGAND programme written by Munson & Rodbard [25], as described previously [17].

Materials

EGF (receptor grade) was obtained from Collaborative Research Corp. (Waltham, Mass.). [^{125}I]EGF was obtained from New England Nuclear (Boston, Mass.).

RESULTS

Effect of Extracellular Ca^{2+} upon Cornified Envelope Formation and Cell Morphology

The ability of keratinocytes to form a cornified envelope provides a highly specific indicator for keratinocyte differentiation. Usually, only a small proportion of the cells form spontaneously cornified envelopes in culture; however, in the presence of the Ca^{2+}-ionophore X537A the cornified envelope formation was greatly enhanced, thus providing a method to study the ability of cornified envelope formation. In media containing normal Ca^{2+} concentrations (1.6 mM), all normal keratinocytes and SVK_{14} cells were able to form cornified envelopes, while this ability decreased progressively in the squamous carcinoma cell lines in the order SCC-12F2 > SCC-15 > SCC-12B2 > SCC-4 (table 1), demonstrating a differential defect in terminal differentiation capacity in these cell lines. Lowering the extracellular Ca^{2+} concentration to 0.06 mM during the culture period, resulted in a ~2-fold decrease in cornified envelope formation in normal keratino-

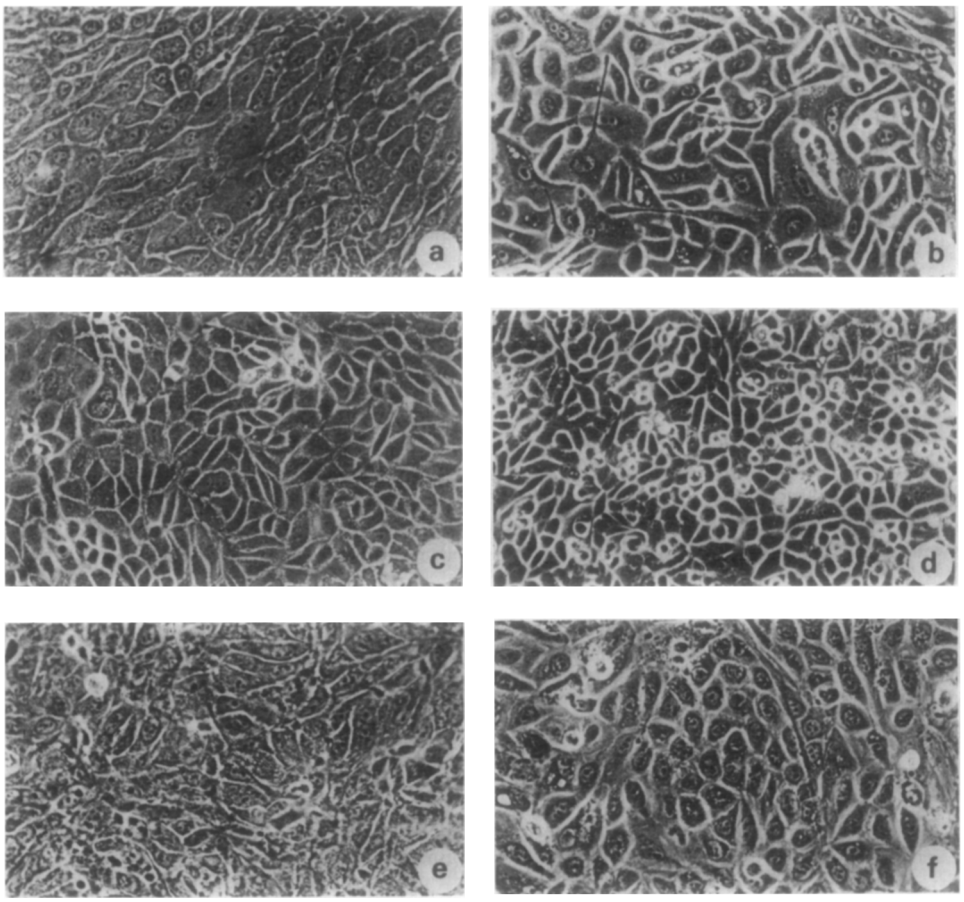

Fig. 1. Effect of extracellular calcium concentration on cell morphology in various human keratinocyte cell lines. Cells were grown either under (*a, c, e, g, i, k*) normal (1.6 mM) external calcium concentrations or under (*b, d, f, h, j, l*) low (0.06 mM) external calcium concentrations as described in Materials and Methods. (*a, b*) Keratinocytes; (*c, d*) SVK_{14}; (*e, f*) SCC-12F2; (*g, h*) SCC-12B2; (*i, j*) SCC-15; (*k, l*) SCC-4.

cytes and SVK_{14} cells, while only a small fraction of the SCC lines were able to form cornified envelopes (table 1).

In addition to large effects of the extracellular Ca^{2+} concentration on cornified envelope formation, dramatic effects were also observed on cell morphology (fig. 1). In low Ca^{2+} medium the size of normal keratinocytes increased and they obtained an irregular cell shape. The increase of the cell body has been established by cell surface area measurements (table 1), showing an 1.6-fold increase as cells were grown to confluency under low Ca^{2+} concentrations. The size of SVK_{14} cells, on the other hand, appeared unchanged under low Ca^{2+} conditions, their surface borders being more clearly defined as compared with normal Ca^{2+} conditions (fig. 1 *C, D*). The cell surface area measured showed only a 1.1-

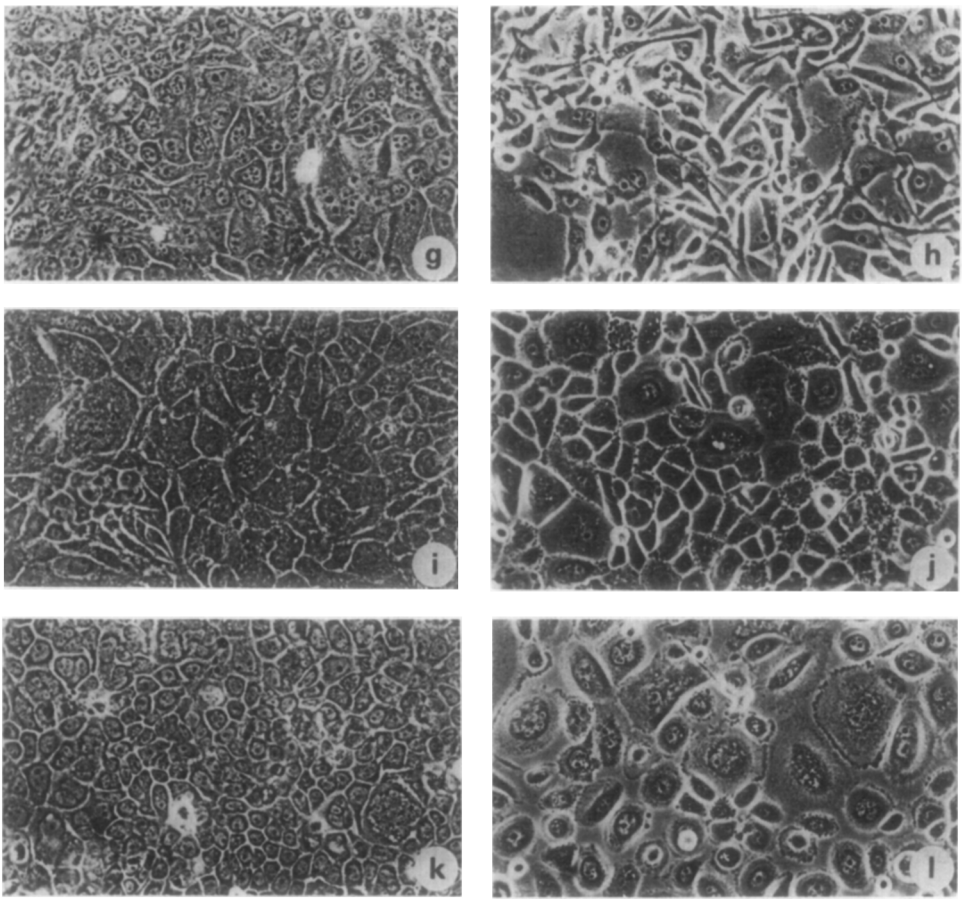

Table 1. *Effect of extracellular calcium concentration on the formation of cornified envelope and cell surface area in various human keratinocyte cell lines*

Cell line	Cornified envelope formation (% of total cell number)		Cell surface area (μm²/cell)	
	0.06 mM Ca^{2+}	1.6 mM Ca^{2+}	0.06 mM Ca^{2+}	1.6 mM Ca^{2+}
Keratinocytes	55	100	2 915	1 875
SVK_{14}	45	100	1 717	1 519
SCC-12F2	45	70	2 219	2 320
SCC-12B2	7	24	3 297	2 333
SCC-15	10	36	2 666	2 371
SCC-4	2	10	2 800	2 063

Measurement of cornified envelope formation has been carried out as described under Materials and Methods. The cell surface area was determined as described in detail previously [20].

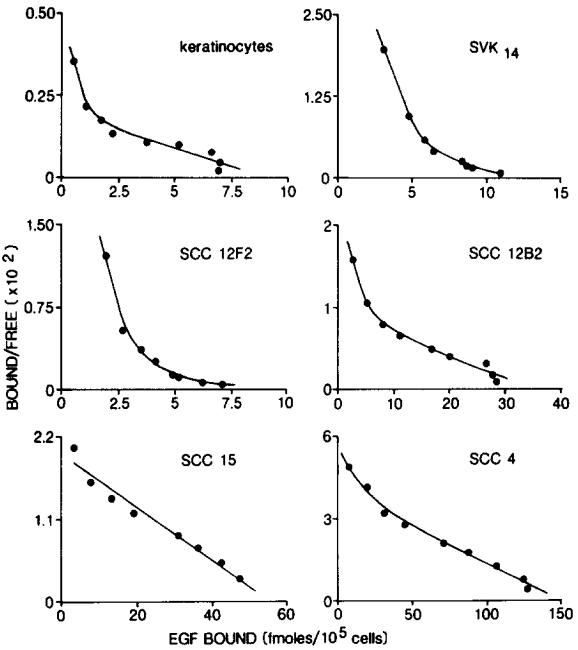

Fig. 2. EGF binding in various cell lines cultured under normal external Ca^{2+} concentrations. Cells were grown under normal (1.6 mM) external calcium concentrations as described in Materials and Methods. [^{125}I]EGF-binding was performed at room temperature during a 2-h incubation period. The binding was determined as described previously [17].

fold increase upon growth under low Ca^{2+} conditions (table 1). A similar morphological difference was observed in the SCC cell lines (fig. 1). The cell surface area increased largely in SCC-4 and SCC-12B2 cells during growth under low Ca^{2+} conditions, in contrast to SCC-12F2 and SCC-15 (table 1).

EGF Binding in Cells Grown under Normal Ca^{2+} Conditions

EGF binding was assayed on monolayer cells at room temperature in normal keratinocytes, SVK_{14}, SCC-12F2, SCC-12B2, SCC-15 and SCC-4 cells, as described under Materials and Methods. A steady state of EGF binding was obtained within 2 h of incubation in the presence of various concentrations of [^{125}I]EGF in all cell lines, but minor differences among the various cell lines were observed in the time period required to reach this steady state.

Subsequently, the concentration dependency of EGF binding was measured in all cell lines, using a 2-h incubation period in the presence of varying concentrations of EGF at room temperature. The binding data were subjected to Scatchard analysis using a computer program LIGAND, written and described by Munson & Rodbard [25]. LIGAND is essentially a non-linear model fitting program, in which statistical comparisons and goodness-of-fit tests are performed [25]. The Scatchard graphs of the various cell lines are shown in fig. 2. A best fit through the data points revealed two classes of binding sites in all cell lines, except for the SCC-15 cells. The presence of two classes of EGF-binding sites has been reported in a variety of epithelial cell lines, such as HeLa, KB, A431 cells and mouse

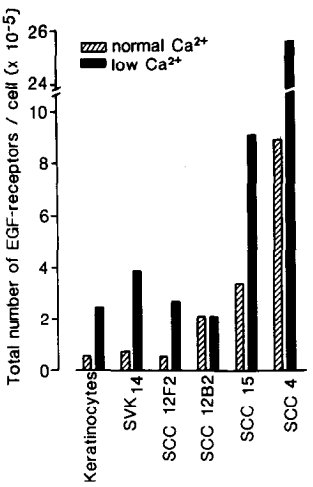

Fig. 3. Effect of external calcium concentrations upon total number of EGF-receptors/cell. Cells were grown under normal (1.6 mM) or low (0.06 mM) external calcium concentrations as described in Materials and Methods. The total number of EGF-binding sites/cell was obtained from Scatchard analysis as shown in figs 2 and 4 respectively.

mammary epithelial cells [26–28]. A large difference between the various cell lines was observed with regard to the number of EGF-binding sites (fig. 3). As the cells showed a decreasing ability to differentiate, the total number of EGF-binding sites increased considerably, from approx. 50000 in normal keratinocytes up to 870000 in SCC-4 (fig. 3). The different number of EGF-binding sites is caused predominantly by the number of low-affinity binding sites, as shown in table 2. In addition a large variation in the number of high-affinity binding sites was found in the various cell lines, although these numbers could not be related to the extent of keratinocyte differentiation (table 2). Since the cell surface area was found to differ between the various cell lines, expression of the density of EGF receptors might provide more reliable information. However, the differ-

Table 2. *EGF-binding characteristics in various human keratinocyte cell lines, cultured under normal Ca^{2+} concentrations*

	High-affinity class				Low-affinity class			
Cell line	k_d (nM)	Maximal binding (fmoles/ 10^5 cells)	Receptors/ cell	Receptors/ μm^2	k_d (nM)	Maximal binding (fmoles/ 10^5 cells)	Receptors/ cell	Receptors/ μm^2
Keratinocytes	0.007	0.3	1 800	0.95	5.21	8.8	53 000	28.22
SVK_{14}	0.11	4.8	29 000	19.04	4.02	7.1	43 000	28.07
SCC-12F2	0.10	2.9	17 000	7.43	5.35	5.5	33 000	14.26
SCC-12B2	0.04	1.6	9 700	4.17	3.91	32.1	193 000	82.76
SCC-15					2.80	55.0	331 000	139.73
SCC-4	0.53	5.2	32 000	15.58	4.01	143.7	866 000	419.53

The binding characteristics were determined using the data presented in fig. 2 with a computer programme LIGAND as described under Materials and Methods.

Table 3. *EGF-binding characteristics in various human keratinocyte cell lines, cultured under low calcium conditions*

Cell line	k_d (nM)	Maximal binding (fmoles/10^5 cells)	Receptors/cell	Receptors/μm^2
Keratinocytes	0.84	40.4	243 000	83.48
SVK_{14}	2.01	64.1	386 000	224.89
SCC-12F2	1.76	44.0	265 000	119.31
SCC-12B2	1.16	34.3	206 000	62.59
SCC-15	4.65	152.4	918 000	344.32
SCC-4	1.77	425.8	2565 000	915.93

The binding characteristics were determined using the data presented in fig. 4, using a computer programme LIGAND as described under Materials and Methods.

ences observed in number of EGF receptors in the various cell lines was not due to increases in cell surface area, as shown in table 2. The apparent dissociation constant (k_d) of the low-affinity class of binding sites was approximately equal in all cell lines, ranging between 2.8 and 5.4 nM. A relative larger difference was found in the k_d of the high-affinity class, the highest affinity was measured in normal keratinocytes (0.007 nM) and the lowest affinity in SCC-4 cells (0.53 nM). Furthermore, a clear relationship of the k_d of the high-affinity class with the extent of keratinocyte differentiation is not apparent, although normal keratinocytes showed an extremely high affinity.

EGF Binding in Cells Grown under Low Ca^{2+} Conditions

Cells were grown to confluency and incubated in media containing 0.06 mM Ca^{2+} for 10 days. EGF binding in these cells was at steady state within 2 h of incubation in the presence of [^{125}I]EGF at room temperature, as for normal Ca^{2+} conditions. The concentration dependency of EGF binding was subsequently determined and the data analysed and plotted according the Scatchard method as described above. The most obvious difference with the data shown in fig. 2 is the absence of a high-affinity class of EGF-binding sites in all cell lines, as all data points could be fitted to straight lines in a Scatchard graph (fig. 4). The total number of binding sites was significantly increased, as compared to the data in fig. 3, in all cell lines, except for SCC-12B2 (fig. 3). Normal keratinocytes, SVK_{14}, and SCC-12F2 contained approx. 200 000–350 000 sites/cell, this number being a 4–5-fold increase as compared with the number of binding sites under normal Ca^{2+} conditions. The increase of EGF-binding sites was about 2.8-fold in SCC-15 and SCC-4 cells (fig. 3). As shown in table 3, the increase of EGF-binding sites under low Ca^{2+} conditions was not due to increased cell surface area, since also the density of EGF-binding sites increased. The k_d ranged between 0.8 and 4.7 nM, and was fairly similar to the k_d of the low-affinity class of cells cultured under normal Ca^{2+} conditions. These findings suggest that the low-affinity class

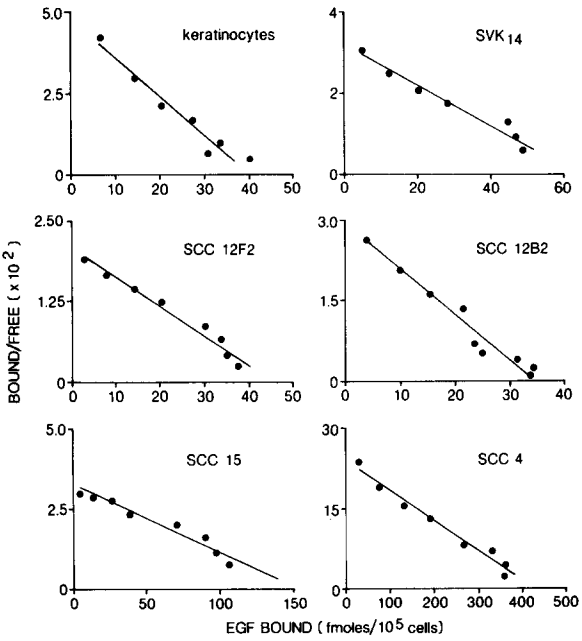

Fig. 4. EGF binding in various cell lines cultured under low external Ca^{2+} concentrations. Cells were grown under low (0.06 mM) external calcium concentrations as described in Materials and Methods. [^{125}I]EGF binding was performed at room temperature during a 2 h incubation period in medium containing 0.06 mM calcium. The binding was determined as described previously [17].

is the only class which is present in cells grown under low Ca^{2+} conditions. Because the high-affinity site could not be detected under low Ca^{2+} conditions and the k_ds under low Ca^{2+} conditions were similar to those of the low-affinity class under normal Ca^{2+} conditions, it could be argued that under low Ca^{2+} conditions the high-affinity class has not been detected due to the dramatic increase in the number of low-affinity sites. In this respect it should be noted that no difference in number of binding sites was detected in SCC-12B2 cells under normal and low Ca^{2+} conditions (fig. 3), while also in this cell line the high-affinity class was absent under low Ca^{2+} conditions (fig. 4). These findings suggest, therefore, that culturing the cells in low external Ca^{2+} results not only in an increase in EGF-receptor number per cell but in a fundamental change in EGF-binding characteristics.

Reversibility of Ca^{2+} Effect on EGF Binding

The effects of the external Ca^{2+} concentration on EGF binding in normal keratinocytes have been shown to be reversible [11]. The same phenomenon has been observed in the cell lines used in this study (fig. 5). The cells were cultured to confluency in media with a low Ca^{2+} concentration, followed by a shift to a normal Ca^{2+} concentration. The EGF binding was assayed using 10 ng/ml [^{125}I]EGF and expressed as the percentage of binding in cells grown under continuous low Ca^{2+}. As shown in fig. 5*A*, normal keratinocytes showed a rapid loss of EGF binding, the EGF-binding characteristics were back to normal within 1 day, the half-maximal effect obtained in less than 4 h. SCC-12F2 cells also

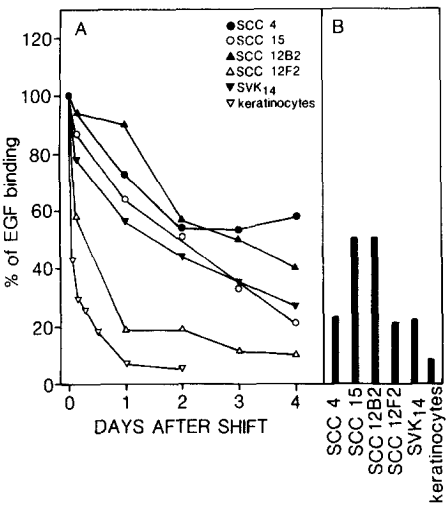

Fig. 5. EGF binding after a shift from low to normal external Ca^{2+} concentrations. (*A*) Cells were grown to confluency and cultured then on low (0.06 mM) external Ca^{2+} concentrations. Subsequently, the medium was replaced for normal Ca^{2+}-containing medium and at the times indicated, the number of cells and EGF binding (using 10 ng/ml EGF) were determined at various time intervals as indicated. The EGF binding is presented as % of binding at low Ca^{2+} concentrations, each point being corrected for cell number. ▽–▽, Normal keratinocytes; ▼–▼, SVK_{14}; △–△, SCC-12F2; ▲–▲, SCC-12B2; ○–○, SCC-15; and ●–●, SCC-4. (*B*) EGF binding at normal Ca^{2+} concentrations as percentage of binding under low Ca^{2+} concentrations using 10 ng/ml EGF. Data being obtained from figs 2 and 4.

exhibited a rapid loss of EGF binding, the half-maximal effect being obtained within 12 h, while in SCC-12B2, SVK_{14}, SCC-15 and SCC-4 the half-maximal effect was obtained in approx. 2 days (fig. 5*A*).

Subsequently EGF binding under continuous normal Ca^{2+} conditions was expressed as the percentage of binding under low Ca^{2+} conditions using an EGF concentration of 10 ng/ml from the data presented in figs 2 and 4 respectively, and these values were compared to those obtained after a Ca^{2+} shift. As shown in fig. 5*B*, the EGF binding under continuous normal Ca^{2+} concentrations was in reasonable agreement with the binding after a shift from low to normal Ca^{2+} after 4 days (fig. 5*A*). Similar data were obtained using either 1 ng/ml or 50 ng/ml EGF (data not shown). Of particular interest is the decrease of EGF binding after a Ca^{2+} shift in SCC-12B2 cells, since no drastic changes were observed in the total number of EGF-binding sites under normal and low Ca^{2+} conditions (fig. 3). As shown in fig. 2, SCC-12B2 cells contain two classes of binding sites in the presence of normal Ca^{2+} conditions, while only one class could be detected under low Ca^{2+} conditions. The number of EGF receptors occupied at a particular EGF concentration is the result of the affinities and the number of binding sites. In SCC-12B2 cells the number of EGF receptors occupied at 10 ng/ml EGF appears to be lower under normal Ca^{2+} conditions than under low Ca^{2+} conditions, although the maximal number of binding sites is similar.

DISCUSSION

The establishment of various squamous carcinoma cell (SCC) lines [20], with a varying degree of defect in terminal differentiation, offered an attractive model system to study the keratinocyte differentiation process. Using these cell lines and in addition also normal keratinocytes and SV40-transformed keratinocytes

(SVK_{14}), we have extended previous studies demonstrating that during keratinocyte differentiation the ability of cells to bind EGF is decreasing. The number of EGF-binding sites of normal keratinocytes under normal and low external Ca^{2+} concentrations, are comparable to that obtained by O'Keefe & Payne [11], although these authors did not observe a high-affinity EGF-binding class under normal Ca^{2+} concentrations. A negative correlation was established between the ability of the various cell lines to form cornified envelopes and the number of EGF-binding sites. Furthermore, it was shown that the external calcium concentration played an important role in keratinocyte differentiation, and that low Ca^{2+} conditions resulted in a marked reduction in capability of all cell lines used to form Ca^{2+} ionophore-induced cornified envelopes. In agreement with these findings an increase was also found in the number of EGF-binding sites in all cell lines, except for SCC-12B2, under these conditions. This increase was not due to increased cell surface area, as was shown by the calculation of the EGF receptor density. The effect of the external Ca^{2+} concentration was most pronounced in the most differentiated cells, normal keratinocytes, SVK_{14} and SCC-12F2, and much less in SCC-15 and SCC-4 cells. Finally, the effects of the external Ca^{2+} concentration on EGF receptor expression were partially reversible within 4 days, the highest reversibility occurring in normal keratinocytes. Altogether, these results demonstrate the relationship between EGF receptor expression and the extent of keratinocyte differentiation.

An intriguing question remaining is directed to the mechanism of regulation of EGF-receptor expression. A clue to this end has probably been obtained recently in differentiating N1E-115 neuroblastoma cells. In these cells neurite outgrowth is associated with a loss of EGF receptors in accordance with a gradual decline in mitogenic stimulability. The loss of EGF receptors is accompanied by an increase in cholesterol biosynthesis and plasma membrane cholesterol content, and thus a decrease in plasma membrane lipid fluidity. In addition, the loss of EGF receptors could be modulated experimentally by inhibition of cholesterol synthesis, which suggests a causal relationship between EGF-receptor expression and membrane lipid fluidity [29–31]. In this respect, a number of recent studies [18–20] on the cholesterol synthesis in normal keratinocytes, SVK_{14} and various SCC lines is of particular importance. In these experiments the LDL-induced regulation of cholesterol synthesis was studied, as well as the metabolism of LDL by assessing its binding, internalization and degradation. Comparison with normal skin fibroblasts showed that most of the cell lines, e.g. normal keratinocytes, SVK_{14}, SCC-12F2, SCC-15, SCC-12B2 and SCC-4, exhibited a defective response to changes in extracellular lipoprotein concentrations. Both the inducibility of cholesterol synthesis in cells deprived of extracellular sources of cholesterol and the LDL-induced suppression of intracellular cholesterol synthesis in cells preincubated in medium supplemented with lipoprotein-deficient serum showed the following sequence: fibroblast > SCC-4 > SCC-15 > SCC-12F2 ~ SCC-12B2 > SVK_{14} ~ normal keratinocytes. Furthermore, for all tested SCC lines, except

SCC-12B2, a good correlation was consistently observed between the degree of LDL-induced suppression of cholesterol synthesis and the decreasing ability of cells to differentiate into squames. These correlations were also observed by changes of the extracellular Ca^{2+} concentrations in normal keratinocytes.

The similarity in the above-mentioned effects on cholesterol synthesis and LDL responsiveness and/or the EGF-receptor expression in relation to keratinocyte terminal differentiation suggest a common mechanism in the regulation of the expression of some membrane receptors, which are internalized via the same endocytotic pathway [32]. Furthermore, it is suggested that the effects of the external Ca^{2+} concentration on the expression of EGF and LDL receptors in keratinocytes is a regulation on existing receptors rather than regulation on metabolic level. This suggestion originates from the observations that (1) in normal keratinocytes the reversibility of EGF- and LDL-receptor expression after a shift of the external Ca^{2+} concentration occurs within hours, while in most cells the EGF-receptor half-life has been reported to be around 20 h in the absence of EGF [33]; (2) a striking difference was observed in EGF-receptor characteristics in SCC-12B2 cells under low and normal Ca^{2+} concentrations, without a significant effect upon the total number of EGF-binding sites; and (3) the changes in EGF-receptor expression were accompanied by changes in cholesterol synthesis, both as related to the external Ca^{2+} concentration, and in the various cell lines used.

An interesting phenomenon is the apparent disappearance of the high-affinity class in cells cultured under low Ca^{2+} conditions. At present we cannot discriminate between an apparent or a real loss, except for the SCC-12B2 cell line.This cell line exhibited more or less the same receptor number under low and normal Ca^{2+} conditions, but did also loose the high-affinity class of binding sites during culturing on low Ca^{2+}. Therefore in this cell line, decreasing Ca^{2+} concentrations results in a drastic decrease of the high-affinity receptors and this might be true for the other cell lines as well.

The results presented here and previously [18–20] allow the following conclusions (1). The SCC lines in combination with normal keratinocytes and SVK_{14} cells provide an attractive system to study various aspects of keratinocyte differentiation in vitro. (2) EGF-receptor expression is strongly related to keratinocyte differentiation, both phenomena being dependent upon the extracellular Ca^{2+} concentration. (3) EGF-receptor expression is related to the ability of cells to differentiate. (4) The data suggest a relationship between receptor expression and the physico-chemical properties of the plasma membrane.

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