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Molecular and functional characterization of sodium–hydrogen exchanger in skin as well as cultured keratinocytes and melanocytes[1]

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Abstract

The sodium–hydrogen (Na^+/H^+) exchanger is one of the few transporter proteins involved in the regulation and maintenance of intracellular pH and cell volume in most eukaryotic cell types. The current study investigates the expression of isoforms of the Na^+/H^+ exchanger (NHE) in human skin and in cultured keratinocytes, melanocytes, and melanoma cells by reverse transcription–polymerase chain reaction (RT–PCR), immunohistochemical analysis and functional studies. Neonatal foreskins were used to isolate RNA from epidermis and dermis, and to initiate cultures of keratinocytes and melanocytes. RT–PCR on RNA isolated from epidermis, dermis, keratinocytes, melanocytes and melanoma cells using PCR primers specific for NHE-1 yielded a 463 bp PCR product. RT–PCR performed using primers specific for NHE isoforms 2, 3, 4 and 5 did not yield any products. Western blotting analysis (of keratinocyte and melanocyte cell cultures) and indirect immunohistochemistry on neonatal foreskin, keratinocytes, melanocytes and melanoma cells using a NHE-1-specific polyclonal antibody demonstrated NHE-1 expression at the protein level. Physiological regulation of intracellular pH using a pH-sensitive dye, BCECF, detected an amiloride-sensitive NHE activity in human keratinocyte, melanocyte and melanoma cell cultures. These results indicate that cultures of human keratinocytes and melanocytes established from human skin and melanoma cells express the NHE-1 isoform of the sodium–hydrogen exchanger.

Keywords: Melanocyte; Keratinocyte; Na^+/H^+ exchanger; Melanoma; (Skin)

1. Introduction

Although there is a plethora of studies evaluating the effect of skin-surface pH in cutaneous health and disease [1], information on the mechanisms involved in the regulation of intracellular pH of cells that make up the layers of skin is lacking. Similar to other eukaryotic cells, the regulation of intracellular pH of cells of the dermis and viable epidermis is necessary to maintain normal metabolic functions [2]. Intracellular pH in most cells is regulated by transport systems including the H^+-ATPase, Na^+/HCO_3^+ exchanger, $C1^-/HCO_3^-$ exchanger, H^+/lactate symporter and the Na^+/H^+ exchanger [3]. In the present study, we characterized the expression of the Na^+/H^+ exchanger isoform(s) and pH regulation

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in cultured keratinocytes and melanocytes derived from cutaneous epidermis as well as melanoma cells.

The sodium–hydrogen exchangers (NHEs) constitute a family of integral membrane antiporters that regulate intracellular pH by effectively extruding H^+ ions from the intracellular compartment in exchange for external sodium [4]. It is an electroneutral antiporter, the activity of which is inhibited by micromolar concentrations of amiloride [5,6]. To date, six NHE isoforms (NHE 1–6) have been identified [7–12]. NHE (1–5) are proteins with molecular mass between 80 and 90 kDa and share approximately 35–60% amino acid identity and are derived from distinct genes [4]. NHE-1 is present in most tissues and cells and is thought to maintain 'housekeeping' functions including maintenance of cell pH and cellular volume. NHE-2, NHE-3 and NHE-4 have a more specific pattern of expression and are found preferentially in various regions of the gastrointestinal tract and the kidneys [8,9,11]. NHE-5 is expressed in brain, spleen, testis and skeletal muscle [10] while NHE-6 is expressed in the mitochondria [12]. However, expression of the NHE isoforms in human skin has not been thoroughly investigated.

In the present study, the NHE isoform(s) expressed in skin as well as cultured keratinocytes, melanocytes and melanoma cells was determined by (1) reverse transcription–polymerase chain reaction (RT–PCR) using isoform-specific primers, (2) indirect immunohistochemistry and (3) use of a pH-sensitive dye to measure intracellular pH changes associated with the Na^+/H^+ exchanger. Only NHE-1 isoform of the NHE was present and functional in these epidermal cells.

2. Materials and methods

2.1. Processing of neonatal foreskins and establishment of cell cultures

Neonatal foreskins were obtained after routine circumcisions from the clinics at the University of Cincinnati. The foreskins were washed with ice-cold Hank's buffered salt solution (HBSS) (Biowhittaker, Walkersville, MD). The skin was incubated in Thermolysin (Sigma Chemical Co., St. Louis, MO) for 18 h at 4°C and then the epidermis separated from dermis using sterile forceps. The epidermis layer was incubated in HBSS containing 0.025% trypsin for 10 min. After a brief vortex, the cell suspension was centrifuged at 1500 rpm in an IEC CRU-5000 centrifuge for 3 min. The supernatant was discarded and the pellet resuspended in fetal bovine serum. Equal portions of the suspension were used for initiate keratinocytes and melanocytes cultures. Keratinocytes were grown in Medium 154 supplemented with 0.2 mM calcium, human keratinocyte growth supplements (Cascade Biologicals, Portland, OR) and 1% antibiotic/antimicotic solution (Gibco BRL, Grand Island, NY). Melanocyte cell cultures were grown in MCDB 153 (Sigma) containing 4% fetal calf serum, 5% bovine serum, 0.22 g/L 3-isobutyl-1-methylxanthine, 50 μg/L 12-*O*-tetradecanoyl-phorbol-12-acetate, 0.6 mg/L basic fibroblast growth factor, 5 mg/L insulin, 1 mg/L vitamin E, 1 mg/L transferrin and 1% antibiotic/antimicotic solution (Gibco BRL). Primary melanoma cell lines (WM 39, WM 115) and metastatic melanoma cell lines (WM 1341D, WM 1205 Lu) (a kind gift from Dr Meenhard Herlyn, Wistar Institute, Philadelphia, PA) were grown in tumor media consisting of MCDB 153 (Gibco BRL), 20% Leibovitz (L-15) Media (Gibco BRL), supplemented with 5 mg/L insulin, 10% fetal bovine serum and 1% antibiotics (Gibco BRL).

2.2. RNA isolation

Neonatal foreskins were washed with ice-cold HBSS buffer. The skins were separated into dermis and epidermis as described above. Established melanocytes and keratinocyte cultures were detached from flasks by trypsinization. Total RNA was isolated from the epidermis, dermis, cultured keratinocytes, cultured melanocytes, melanoma cells, rat kidney and rat brain by the one-step procedure of Chomczynski and Sacchi [13] using TRI Reagent (MRC Biologicals, Cincinnati, OH).

2.3. Reverse transcription and polymerase chain reaction

cDNA was synthesized from total RNA using 200 U of Superscript RNase H_Reverse Transcriptase II (Gibco BRL, Gaithersburg, MD). Each 20-μl reaction mixture contained 5 mM $MgCl_2$, 1×PCR

buffer, 1 mM each deoxyribonucleotide, 2 U of ribonuclease (RNase) inhibitor and 2.5 μM Oligo(dT)$_{12-18}$ primer (Gibco BRL). Reverse transcription was carried out at 42°C for 30 min in a Perkin–Elmer thermal cycler. PCR was performed in 100 μl mixture containing 2 mM $MgCl_2$, 1×PCR buffer, 1 mM each deoxyribonucleotides, 20 μM each (of forward and reverse PCR primers) and 2.5 U of Taq polymerase (95°C, 1 min; 60°C, 1 min; 72°C, 2 min; 35 cycles)] followed by 72°C, 15-min extension using NHE isoform-specific primers. The NHE isoform-specific PCR primers were designed based on published shared human and rat sequences to amplify PCR fragments of each human NHE isoform as described previously [14], and were synthesized at Marshall University DNA Core facility (Huntington, WV). PCR products were analyzed by size fractionation in a 2% agarose gel stained with ethidium bromide.

2.4. Western blot analysis

Confluent flasks containing cultured keratinocytes and melanocytes were used for determination of protein expression of the NHE-1 isoform of the sodium–hydrogen exchanger. Cell lysates were prepared using RIPA buffer [15] containing protease inhibitors methylsulfonyl fluoride (200 mM), aprotinin (10 mM) and leupeptin (10 μg per ml). Equal amounts of protein (50 μg) were loaded on each lane and separated on a 8% polyacrylamide gel by electrophoresis. Following transblotting onto nitrocellulose membranes, the membranes were incubated with NHE-1-specific affinity purified polyclonal antibody (1:300 dilution); the generation and characterization of this antibody has been described in detail previously [16,17]. The membranes were then incubated with alkaline phosphatase-conjugated anti-rabbit IgG (Amersham; 1:3000 dilution). The immunoreactive bands were detected using the BCIP/NBT Phosphatase Substrate System (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

2.5. Immunostaining of skin and cultured cells

Human neonatal foreskin and rat kidneys were embedded in OCT compound and snap-frozen in liquid nitrogen. Sections (8 μm thick) were made using a Tissue-Tek II Cryostat. A three-step indirect immunoperoxidase or immunofluorescence staining was performed using a polyclonal NHE-1 immune serum (a generous gift from Dr E.B. Chang, University of Chicago, IL) that recognizes the carboxy terminus of the NHE-1 isoform [16]. A polyclonal antisera hPEP7 that recognizes human melanocyte-specific gene product, tyrosinase (1:100) (a gift from Drs King and Oetting, University of Minnesota, MN) was used as positive control for melanocytes.

2.5.1. Immunoperoxidase staining

The initial attempt to characterize NHE-1 in skin, keratinocytes, melanocytes and melanoma cells was accomplished by immunoperoxidase staining. Keratinocytes, melanocytes and melanoma cells were subcultured on glass slides and fixed in cold acetone. The cells were washed thrice in phosphate-buffered saline (PBS) (pH 7.4), and incubated in PBS containing 0.03% hydrogen peroxide for 10 min to inactivate internal peroxidase. After a subsequent incubation in 10% normal human serum (NHS) for 1 h, the specimens were exposed to primary antiserum (1:100 dilution in 10% NHS) for an additional hour. The specimens were washed and incubated with the secondary antibody (biotinylated anti-rabbit IgG at 1:200 dilution in 10% NHS). After washing with Tris-buffered saline (pH 7.4), aminoethylcarbazole (AEC) was used as a peroxidase substrate in the presence of 0.03% hydrogen peroxide for development of color.

2.5.2. Immunofluorescence

Melanocytes were subcultured on glass slides and fixed in 5% formaldehyde. Briefly, the cells were incubated with 100% methanol for 3 min, washed thrice in PBS and incubated for 1 h in 10% NHS. Specimens were then incubated in primary antiserum (1:100 dilution in 10% NHS) for 1 h, washed and incubated with the secondary antibody (TRITC anti-rabbit IgG at 1:200 dilution in 10% NHS) for 1 h. After washing with Tris-buffered saline (pH 7.4), the specimens were mounted with a glass coverslip and observed for fluorescence using a Leitz Fluorescence microscope fitted with a TRITC filter.

2.6. Intracellular pH measurement

The intracellular pH in melanocytes, keratinocytes

and melanoma cell cultures melanoma cells grown on coverslips was measured using pH-sensitive dye 2′,7′-biscarboxyethyl-5 (and -6) carboxyfluorescein (BCECF) as described previously [18–20]. To measure pH_i, the cells were incubated in the presence of 5 μM BCECF for 10 min in a solution consisting of 140 mM Tris(hydroxymethyl)aminomethane (TMA-Cl), 0.8 mM K_2HPO_4, 0.2 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes (pH 7.4; solution A). The fluorescence of BCECF was monitored at excitation wavelengths 450 and 500 nm and emission wavelength 525 nm in a thermoregulated (37°C) double-excitation beam spectrofluorometer (PTI double-beam fluorometer, Delta Scan I, Photon Technologies International, Eugene, OR). A calibration curve was generated using the KCI/nigericin technique [21], and solutions of varying pH. The fluorescence ratio at 500/450 nm was utilized to determine intracellular pH values in the experimental groups by comparison with the calibration curve. Acid loading of cells grown on coverslips was achieved via NH_4 pulse [21]. The NH_4Cl-containing solution consisted of 120 mM TMA-Cl, 20 mM NH_4Cl, 0.8 mM K_2HPO_4, 0.2 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes (pH 7.4; solution B). Cell acidification was induced by replacing NH_4Cl-containing solution with solution A. Following acid loading, the initial rate of pH recovery was monitored in the presence of a sodium-containing solution consisting of 140 mM NaCl, 0.8 mM K_2HPO_4, 0.2 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes (pH 7.4; solution C). Inhibition of the initial rates of pH recovery in melanocytes, keratinocytes and melanoma cells was determined in the presence of 100 μM amiloride, a specific inhibitor of the sodium–hydrogen exchanger. Involvement of the Na^+/H^+ exchanger in pH_i recovery was measured using amiloride, a specific inhibitor of the exchanger.

3. Results

3.1. RT–PCR

Total RNA isolated from rat kidney (used as positive control for NHE-1, -2, -3 and -4 isoforms), epidermis/dermis separated from human skin as well as

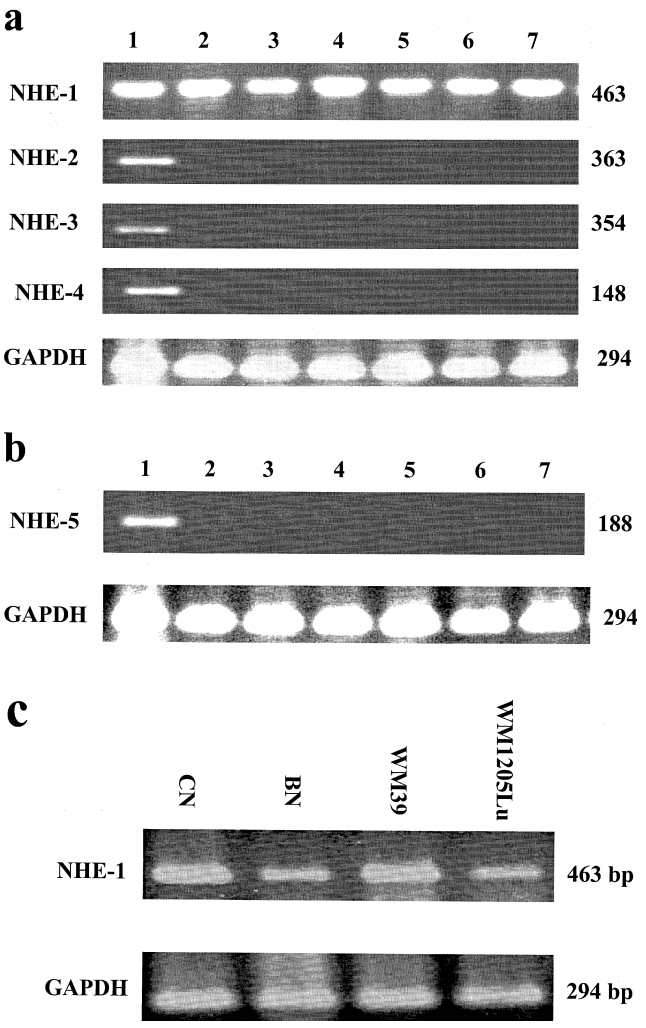

Fig. 1. Reverse transcription–polymerase chain reaction (RT–PCR) analysis of RNA for presence of sodium-hydrogen exchanger isoform(s). RT–PCR was performed on total RNA isolated from skin (lane 2), epidermis (lane 3), dermis (lane 4), keratinocytes (lane 5), melanocytes (lane 6) and melanoma cells (WM1205Lu) (lane 7) as described in Section 2. Rat kidney RNA was used as positive control for NHE-1, -2, -3 and -4 isoform (a, lane 1) and rat brain RNA for NHE 5 isoform (b, lane 1). PCR products were size fractionated on 1.2% agarose gel and visualized by ethidium bromide staining. Number on right side of panel designates the expected size of PCR product. All samples tested yielded a product of expected size for NHE-1 isoform. Only rat kidney RNA yielded a PCR product of expected size for isoforms NHE-2, -3 and -4 (a, lane 1) and rat brain RNA for NHE-5 isoform (b, lane 1). All samples yielded PCR product for GAPDH (a,b, bottom panels). (c) NHE-1 mRNA was detected in normal human melanocytes (derived from Caucasian (CN) and African-American (BN) individual) cultured in melanoma media for 14 days as well as in melanoma cells cultured from a primary lesion (WM39) and a metastatic lesion (WM1205Lu).

cultured keratinocytes, melanocytes and melanoma cells were assessed for the expression of the NHE isoform(s) by reverse transcriptase polymerase chain reaction. Fig. 1a represents ethidium bromide stained 1.2% agarose gel size fractionation of the resulting PCR products. RT–PCR analysis of rat kidney RNA using primers specific for NHE isoforms 1, 2, 3 and 4 yielded product of expected size (Fig. 1a, lane 1), the sequence of which is identical to the published human NHE-1 sequence [14]. A 463 bp PCR product corresponding to NHE-1 isoform was observed in all samples. RT–PCR analysis of skin, epidermis/dermis separated from skin and cultures of keratinocytes, melanocytes and melanoma cells did not yield products of expected sizes for NHE isoforms 2, 3 and 4 (Fig. 1a). RT–PCR of rat brain RNA using primers specific for NHE-5 isoform yielded products of expected size whereas RNA from skin, epidermis and dermis from human skin and cultures of keratinocytes, melanocytes and melanoma cells under identical conditions did not yield any products (Fig. 1b). RT–PCR analysis of all RNA samples for GAPDH yielded product of expected size (Fig. 1a,b, bottom panels).

Melanocytes were cultured in media containing 3-isobutyl-1-methylxanthine and 12-*O*-tetradecanoylphorbol-12-acetate, in contrast to melanoma cells that were cultured in media lacking these growth factors. To facilitate proper comparison of NHE expression between normal melanocytes and melanoma cells, we maintained normal melanocyte cultures in melanoma media for 14 days. RT–PCR of RNA isolated from normal melanocyte cultures maintained in melanoma media did not alter expression of NHE-1 isoform (Fig. 1c).

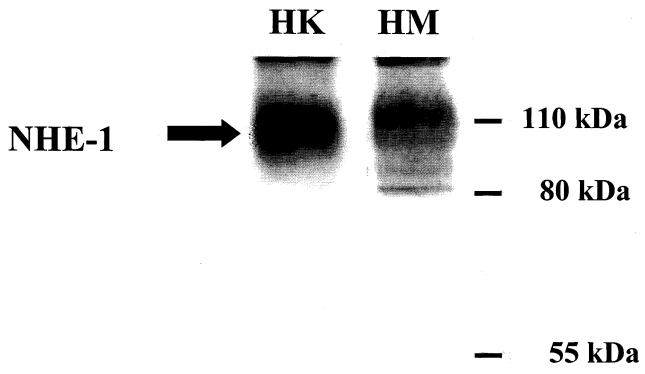

Fig. 2. Western blot analysis for expression of NHE-1 in cultures of keratinocytes and melanocytes. Expression of NHE-1 protein in cell lysates obtained from established cultures of keratinocytes and melanocytes was performed as described in Section 2. As expected, a band of approximately 110 kDa is detected in lanes marked HK (normal human keratinocytes) and HM (normal human melanocytes).

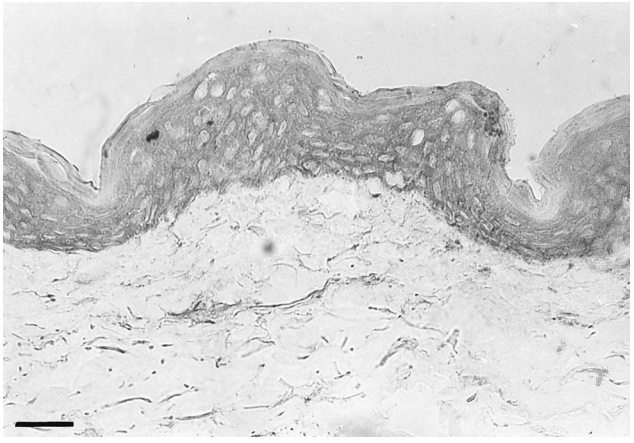

Fig. 3. Immunohistochemical analysis of the expression of NHE-1 isoform in skin. The expression of NHE-1 isoform was detected by applying indirect peroxidase staining of skin as described in Section 2. There is intense staining for NHE-1 isoform observed in the epidermal layers, while no significant staining is observed in the dermis. Scale bar = 50 μm.

3.2. Western blot analysis

Expression of NHE-1 protein in cultures of keratinocytes and melanocytes was determined by western blot analysis. The NHE-1-specific affinity purified polyclonal antibody recognized a single band of ~110 kDa in both keratinocyte and melanocyte cell lysates (Fig. 2) and is consistent with previously published reports [16,17].

3.3. Immunohistology

Initially, expression of the NHE-1 isoform in skin (Fig. 3), cultured keratinocytes, melanocytes, melanoma cells (Fig. 4) was determined using a immunoperoxidase method. Staining for NHE-1 was intense throughout the epidermal layer and minimal within the dermis of the skin (Fig. 3). Although NHE-1 was detected in cultured keratinocytes (Fig. 4B) and melanoma cells (Fig. 4E–H), expression was not apparent in melanocytes (Fig. 4D) probably due to the presence of melanin masking the AEC reaction products. To circumvent this problem, an attempt was

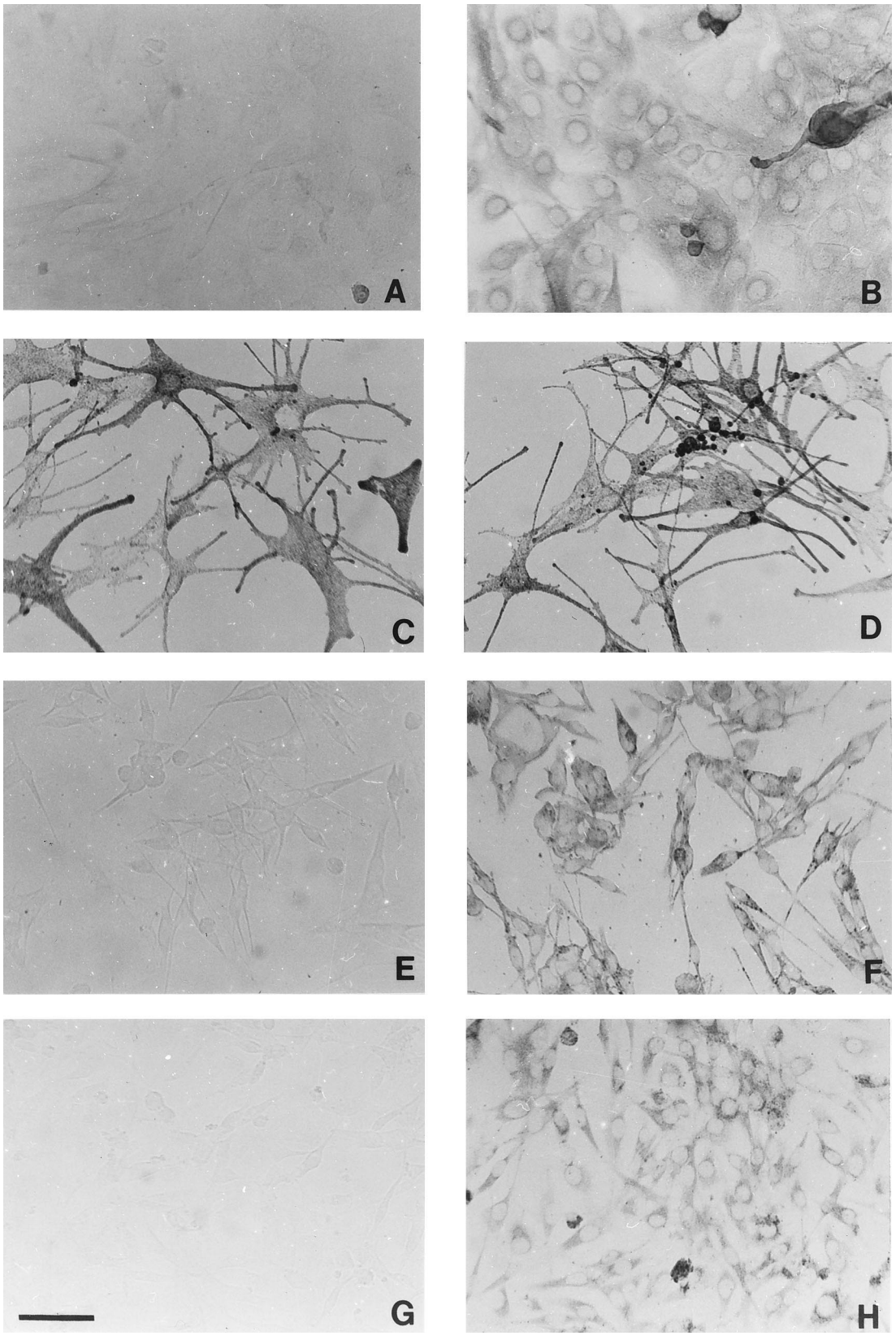
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Fig. 4. Detection of expression of NHE-1 isoform in cultured cells by immunoperoxidase staining. The expression of NHE-1 isoform was detected by applying indirect peroxidase staining of cultured cells as described in Section 2. (A,C) Staining of keratinocyte and melanocyte respectively in the absence of primary NHE-1 antibody recognizing serum. (B,D) Staining of corresponding specimens in presence of NHE-1 recognizing antiserum. (E,F) Staining of primary (WM39) and (G,H) metastatic (WM1205Lu) melanoma cells in absence (E,G) and presence (F,H) of primary NHE-1 antisera. Positive staining was prominent in keratinocytes as well as primary and metastatic melanoma cells but not apparent in melanocytes by immunoperoxidase method of detection. Scale bar = 70 μm.
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made to detect NHE-1 and tyrosinase (as positive control) expression in melanocytes by immunofluorescence using a TRITC-labeled secondary antibody. Uniform staining for NHE-1 was observed in melanocyte cultures of Caucasian (Fig. 5a) and African-American (Fig. 5c) origin. Fig. 5b,d represent expression of tyrosinase in the same melanocyte cultures. No staining was observed in melanocyte cultures incubated in secondary antibody in absence of primary antibody (not shown).

3.4. Intracellular pH measurements

The intracellular pH and functional localization of the NHE-1 isoform on the plasma membrane of cultured keratinocytes, melanocytes and melanoma cells was determined using the pH-sensitive dye, BECEF. Cells were grown on coverslips and assayed for sodium-dependent pH_i recovery from acid load. Fig. 6a–c show a representative pH tracing for the melanocyte, keratinocyte and melanoma cells, respectively. Keratinocytes, melanocytes and melanoma cells demonstrate presence of a functional, amiloride-sensitive, Na^+/H^+ exchanger activity. The rate of pH_i recovery from an acid load (dpH_i/dt) was 0.243 ± 0.01 and 0.213 ± 0.01 pH/min for melanocytes and keratinocytes, respectively (Fig. 6a,b). The pH_i recovery was lower at 0.068 ± 0.006 pH/min for melanoma cells (Fig. 6c). The rate of pH_i recovery was inhibited by a Na^+/H^+ exchanger-specific inhibitor, amiloride at 50–100 μM concentration range, is characteristic for inhibition of NHE-1 activity. In the absence of Na^+, there was no pH_i recovery from cell acidosis, indicating absence of functional H^+-ATPase in melanocytes, keratinocytes and melanoma cells.

4. Discussion

We report the identification and characterization of a transporter protein involved in the regulation of intracellular pH in human skin as well as cultured human keratinocytes, melanocytes and melanoma cells. Intracellular pH in cultured human keratinocytes by pH-sensitive dyes like BCECF and its association to keratinocyte proliferation has been previously documented [22]. Presence of a functional Na^+/H^+ exchanger has been demonstrated in mouse keratinocytes by using a pH-sensitive fluorochrome [23]. In this study, we document that human keratinocytes and melanocytes express a functional, amiloride-sensitive NHE-1 isoform of the sodium–hydrogen exchanger. This is not surprising in light of the fact that (1) the NHE-1 isoform is expressed in virtually all tissues and is thought to fulfill 'housekeeping' functions including maintenance of cytosolic pH [4], (2) the outer layers of the skin undergo significant alterations due to changes in skin surface pH and environment [1,24–26], and (3) there is a pH gradient present between the surface of the skin and the viable epidermis [26,27]. Expression of the NHE would be essential for the differentiating keratinocytes to regulate and maintain its intracellular pH in the presence of acidic extracellular pH found in the upper layers of the skin.

The importance of skin surface pH and its relationship to incidence of diseases such as acute eczema seborrheic dermatitis, atopic dermatitis and xeroderma has been a focus of interest for some time [1,28]. Recently, Ohman and Vahlquist [26] have shown the existence of difference in pH gradient across the stratum corneum in X-linked recessive and autosomal dominant ichthyosis compared to normal skin. We speculate that in diseases like ichthyosis, variations in external pH measured across multiple layers of skin affects the intracellular pH and basic metabolic functions of the keratinocytes present in these layers. Since keratinocytes have high levels of expression of NHE-1 isoform and it is believed that increase in Na^+/H^+ exchanger activity is related to differentiation and cellular prolifer-

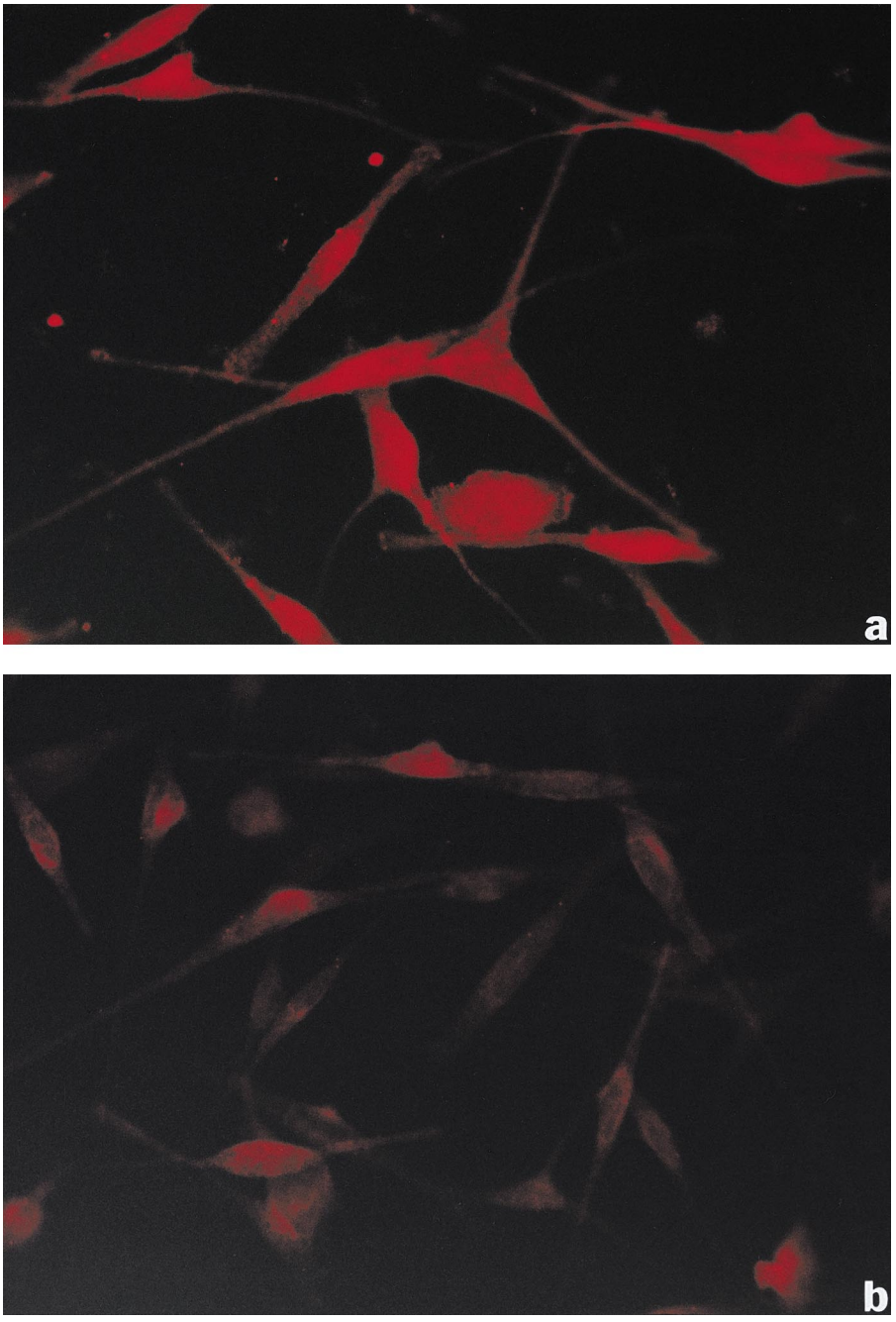

Fig. 5. Detection of expression of NHE-1 isoform in cultured cells by immunofluorescence staining. The expression of NHE-1 isoform was detected by applying indirect immunofluorescence staining of cultured cells as described in Section 2. (a,c) Staining of Caucasian- and African-American-derived melanocytes, respectively, in the presence of primary NHE-1 antibody-recognizing serum. (b,d) Staining of identical melanocyte cultures to detect tyrosinase (using human PEP7 antiserum) as positive control. Staining for NHE-1 and tyrosinase was detectable in both Caucasian- and African-American-derived melanocytes. Scale bar = 20 μm.

ation [3], one can speculate that the Na^+/H^+ exchanger is involved in the hyperproliferation and altered differentiation of the keratinocytes seen in psoriasis [29]. Investigating Na^+/H^+ exchanger activity in keratinocytes is of interest particularly to those involved in manufacturing and testing of personal care products. Items of particular interest are soaps and skin lotions, primarily because soap and detergents

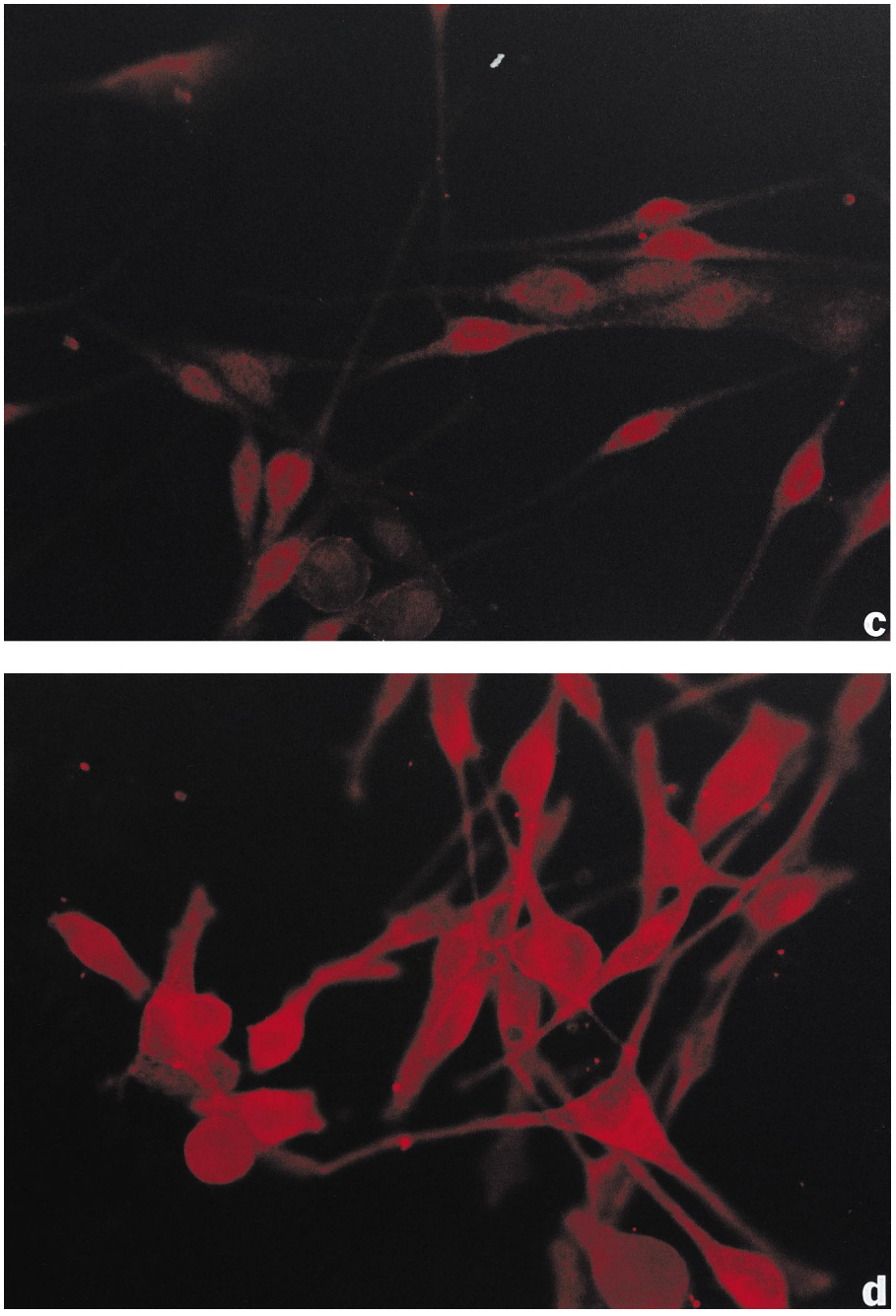

Fig. 5 (*continued*).

affect skin surface pH [30,31] and long-term use of substances like α-hydroxy acids (acidic pH) will affect the basic physiology of keratinocytes.

Amiloride-sensitive Na^+/H^+ exchanger activity was observed in melanocyte cultures both at the mRNA or protein levels. Melanocytes present in the basal layers of the epidermis would require the presence of the Na^+/H^+ exchanger only to extrude H^+ or other acid equivalents that are continuously generated as a byproduct of intermediary metabolism [32] to facilitate maintenance of their intracellular pH. The physiological characteristics of the amiloride-sensitive Na^+/H^+ exchanger present in the melanocyte cultures is consistent with that of the NHE-1

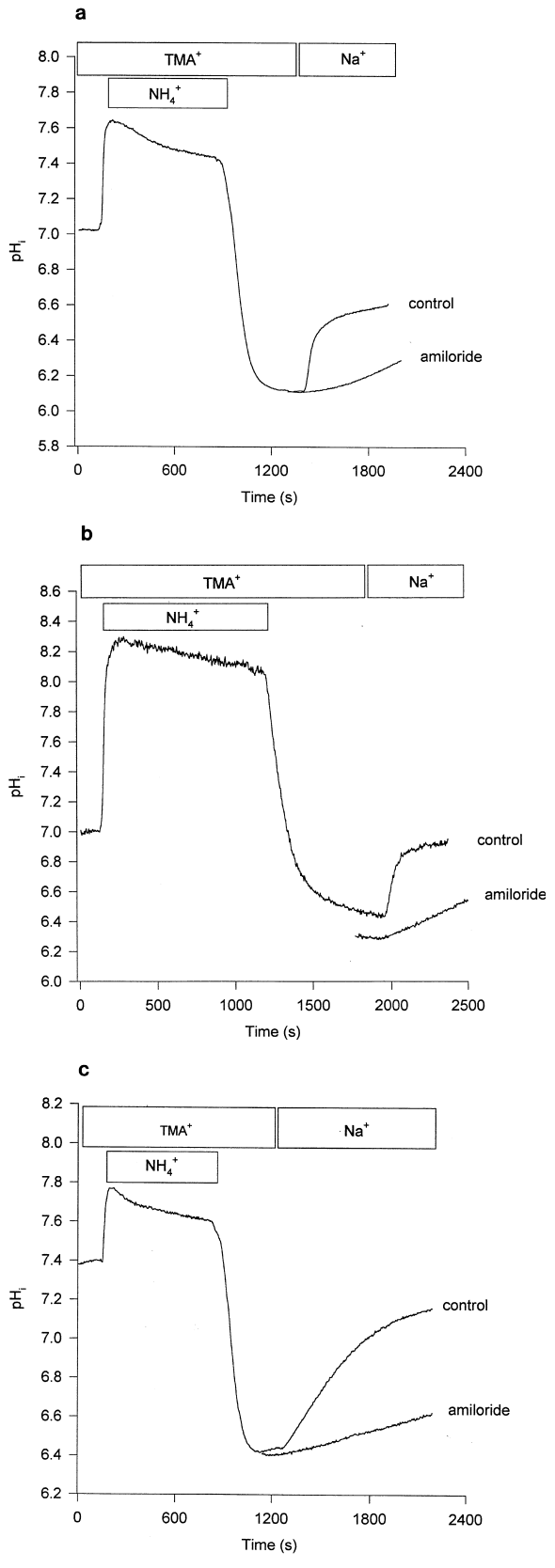

Fig. 6. Sodium-dependent pH_i recovery in keratinocyte, melanocyte and melanoma cells using pH-sensitive dye BCECF. Cells were grown on coverslips, pulsed with ammonium and assayed for presence of Na^+/H^+ exchanger activity as sodium-dependent H^+ extrusion in absence and presence of amiloride (see Section 2 for details). Keratinocytes (a), melanocytes (b) and melanoma cells (c) showed presence of an amiloride-sensitive Na^+/H^+ exchanger activity.
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isoform, suggesting that NHE-1 is the functionally dominant isoform in these cells.

We also demonstrate expression of the NHE-1 isoform in primary and metastatic melanoma cells. During recent years there has been renewed interest in the role of pH in cancer research and process of oncogenesis. Although there are several mechanisms involved in the regulation of cellular ionic homeostasis, the H^+-ATPase and Na^+/H^+ are the two major families of transporters involved in the regulation of intracellular pH in eukaryotic cells. It has been demonstrated that distinct intracellular pH regulatory mechanisms exist in poorly and highly metastatic human melanoma cells [33] and that cytoplasmic pH is important in the modulation of cell growth, proliferation, differentiation, oncogenesis and malignant transformation [34].

Recently it has been demonstrated in the murine model that the Na^+/H^+ (NHE-1 in particular) exchanger is essential for the acidification of the stratum corneum of skin and maintenance of epidermal permeability barrier [35]. An inherent increase in the Na^+/H^+ exchanger activity in cultured skin fibroblast established from diabetic patients has also been demonstrated [36]. Taken together, these studies indicate the importance of the Na^+/H^+ in the maintenance of normal skin function and in disease. The relevance and long-term physiological consequence of altered activity of Na^+/H^+ (and NHE-1) in diabetic skin and other disease conditions needs to be investigated.

The identification of the presence of NHE-1 isoform in keratinocytes, melanocytes and melanoma cells does not preclude the presence of other possible NHE isoforms in these cells. The importance of the Na^+/H^+ exchanger in melanocytes and its role in the regulation of melanocyte function is under investigation. In conclusion, two major cellular components of the skin namely keratinocytes and melanocytes

express the NHE-1 isoform of the sodium–hydrogen exchanger.

Acknowledgements

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