Current Status of Skin Cornification

정수일

㈜녹십자목암연구소

Cornified Cell Envelop (CE)

- CE is a 15 nm thick insoluble layer consists of protein and lipid lamella envelop that is formed under the newly formed lipid membrane in the upper layer of epidermis and keratinizing stratified epithelium.

- CE provides a protective mechanical and chemical barrier of our body.

- CE is maintained by continuous reproduction of inner living epidermal keratinocytes which undergo a process of terminal differentiation and the migrate to the surface as interlocking layers.

Cornified Cell Envelop (CE)

Three area of research data will be discussed in following order.

- Lipid Lamellae
- CE component proteins and assembly
- Crosslinking enzymes (TGases)

대한화장품학회지, 제 25 권, 2 호, 1999

Lipid Lamellae (LL)Formation

- Intercellular LL in the stratum corneum constitute the barrier to water diffusion and may play a role in cohesion between corneocytes.

- LL arise from stacks of lamellar disks that are extruded from the granular cells and fuse edge to edge to form sheets.

Inducible Enzymes in Lipid Lamellar Formation :

- Ceramide : Serine palmitoyl transferase
  Glucose ceramide transferase
  ω-Hydroxylation (P450)
  β-Glucocerebrosidase (+Saposines)

- Cholestrol : HMG CoA reductase
  Cholestrol sulfo transferase
  Steroid sulfatase

Methods used for identifying the crosslinked Proteins in CE.

- For outer membrane side of CE, ceramide lipids were removed by mild alkaline hydrolysis and exposed proteins were analyzed. For cellular side the CE were fragmented and analyzed. Both CE were treated by limited proteolysis and peptides were isolated and sequenced.
- Exposed proteins were identified with respective antibodies.
- Synthetic Lipid Vesicle which is similar to plasma membrane lipids binds involucrin, TGase K, and ceramide, were used for crosslinking of ceramide to protein.

Name	Gene locus	Size (kDa)	Relative abundance in human foreskin CE	Cross-linking sites identified *in vivo*?
Involucrin	1q21 (EDC)	65	2-5%	Yes
Loricrin	1q21 (EDC)	26	80%	Yes
SPRs	1q21 (EDC)	6-26	3-5%	Yes
Cystatin A	3cen-q21	12	2-5%	Yes
Proelafin	20q12-q13	10	<1%	Yes
(Pro)filaggrin	1q21 (EDC)	>400	<1%	Yes
Type II keratins	12q13	56-60	<1%	Yes
Desmoplakin	6p21-tar	330/250	<1%	Yes
Envoplakin	17q25	210	<1%	Yes
Periplakin	16p13.3	195	<1%	Yes
S100 proteins	1q21 (EDC)	12	<1%	No
Annexin I	9q12-q21.2	36	<1%	No

Loricrin

```
  1 msyqkkqptp qppvdcvkts ggggggggtg gggcgffggg gsgggssgsg cgysggggys
 61 gggcgggssg gggggggiggc gggsggsvky sggggssggg sgcfssgggg sgcfssgggg
121 ssgggsgcfs sggggssggg sgcfssgggg fsgqavqcqs yggvssggss gggsgcfssg
181 ggggsvcgys gggsgggsgc gggssggsgs gyvssqqvtq tscapqpsyg ggssggggsg
241 gsgcfssggg ggssgcgggs sgigsgciis gggsvcgggs sgggggggssv ggsgsgkgvp
301 ichqtqqkqa ptwpsk
```

* No tertiary structure(40% glycine)
* Three regions contain crosslinking sites.
* Molecules can be stretched.
* 65 ~ 80% of CE.
* TGase E(TGase 3) likely serves as crosslinking enzyme.
* Fills the inner side of envelop

Involucrin

```
  1 msqqhtlpvt lspalsqell ktvpppvnth qeqmkqptpl pppcqkvpve lpvevpskqe
 61 ekhmtavkgl peqeceqqqk epqeqelqqq hweqheeyqk aenpeqqlkq ektqrdqqln
121 kqleeekkll dqqldqelvk rdeqlgmkke qllelpeqqe ghlkhleqqe gqlkhpeqqe
181 gqlelpeqqe gqlelpeqqe gqlelpeqqe gqlelpeqqe gqlelpqqqe gqlelseqqe
241 gqlelseqqe gqlelseqqe gqlkhlehqe gqlevpeeqm gqlkyleqqe gqlkhldqqe
301 kqpelpeqqm gqlkhleqqe gqpkhleqqe gqleqleeqe gqlkhleqqe gqlehlehqe
361 gqlglpeqqv lqlkqlekqq gqpkhleeee gqlkhlvqqe gqlkhlvqqe gqleqqerqv
421 ehleqqvgql khleeqegql khleqqqgql evpeqqvgqp knleqeekql elpeqqegqv
481 khlekqeaql elpeqqvgqp khleqqekhl ehpeqqdgql khleqqegql kdleqqkgql
541 eqpvfapapg qvqdiqpalp tkgevllpve hqqqkqevqw ppkhk
```

* The outer layer protein of envelop that are in contact with lipid laminae
* Contains unique ten residue TGase substrate sequences that are repeated 39 times in tandem
* Membrane-bound TGase K(TGase 1) specifically catalyzes crosslinking of ceramide and other proteins to nvolucrin

Small Proline-Rich Protein

```
  1 mnsqqqkqpc tpppqpqqqq vkqpcqpppq epcipktkep cqpkvpepch pkvpepcqpk
 61 ipepcqpkvp epcpstvtpa paqqktkqk
```

* 6 X Octapeptide TANDEM REPEATS

```
  1 mssyqqkqtf tpppqlqqqq vkqpsqpppq eifvpttkep chskvpqpgn tkipepgctk
 61 vpepgctkvp epgctkvpep gctkvpepgc tkvpepgctk vpepgytkvp epgsikvpdq
121 gfikfpepga ikvpeqgytk vpvpgytklp epcpstvtpg paqqktkqk
```

* CE precursor ; contains 14 internal octapeptide repeats ; head and tail domains are substrates for transglutaminase-mediated cross-linking

* 4 ~ 5% of CE & linking with Loriclin

Transglutaminases (TGases)

TGases catalyze an acyl transfer reaction involving γ-carboxamide group of pepetide bound glutamine residue to primary amino or hydroxyl group (nucleophile) of aliphatic chain (i.e. lysine, ω-OH ceramide).

- Isopeptide bond [N^{ε}(γ-glutamyl)lysine] formed between peptides induce very stable polymer formation.

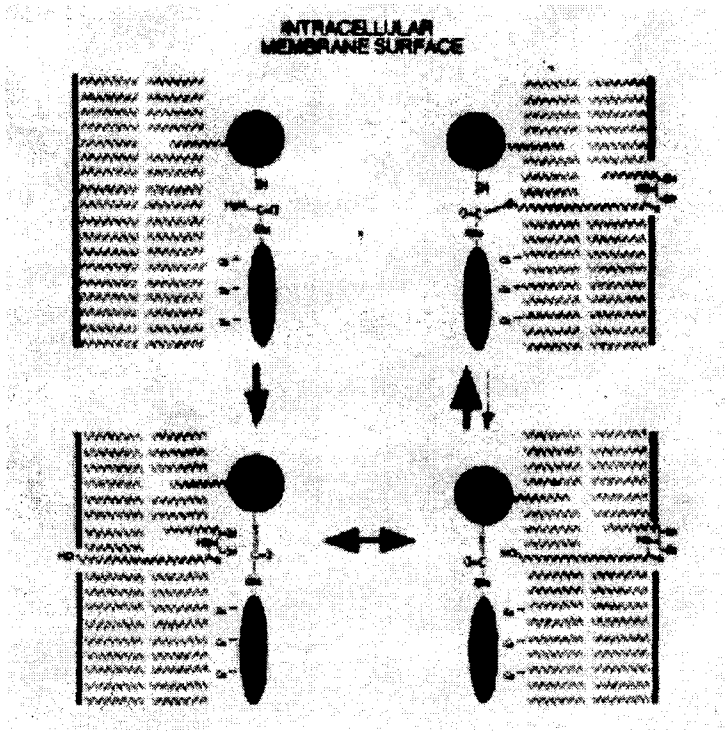
- Ester bond [O^{ω}(γ-glutamyl)ceramide ester] formed between peptide and lipid molecules allow stable lipid-protein lamellae.

Transglutaminase K (TGase 1)

- 106 kD membrane-bound zymogen
- Distributed in keratinocytes(ectodermal), chondrocytes(mesemchy-mal) cellular origin
- Membrane anchored by myristylation and palmitylation
- Activated by proteolysis of two cleavage sites but held together(10, 67 & 30)
- Heavily expressed in granulocyte
- Catalyze the crosslinking reaction of ω–OH group of ceramide to glutamine residue of Involucrin as well as N^{ε}(γ-glutamyl)lysine crosslinks

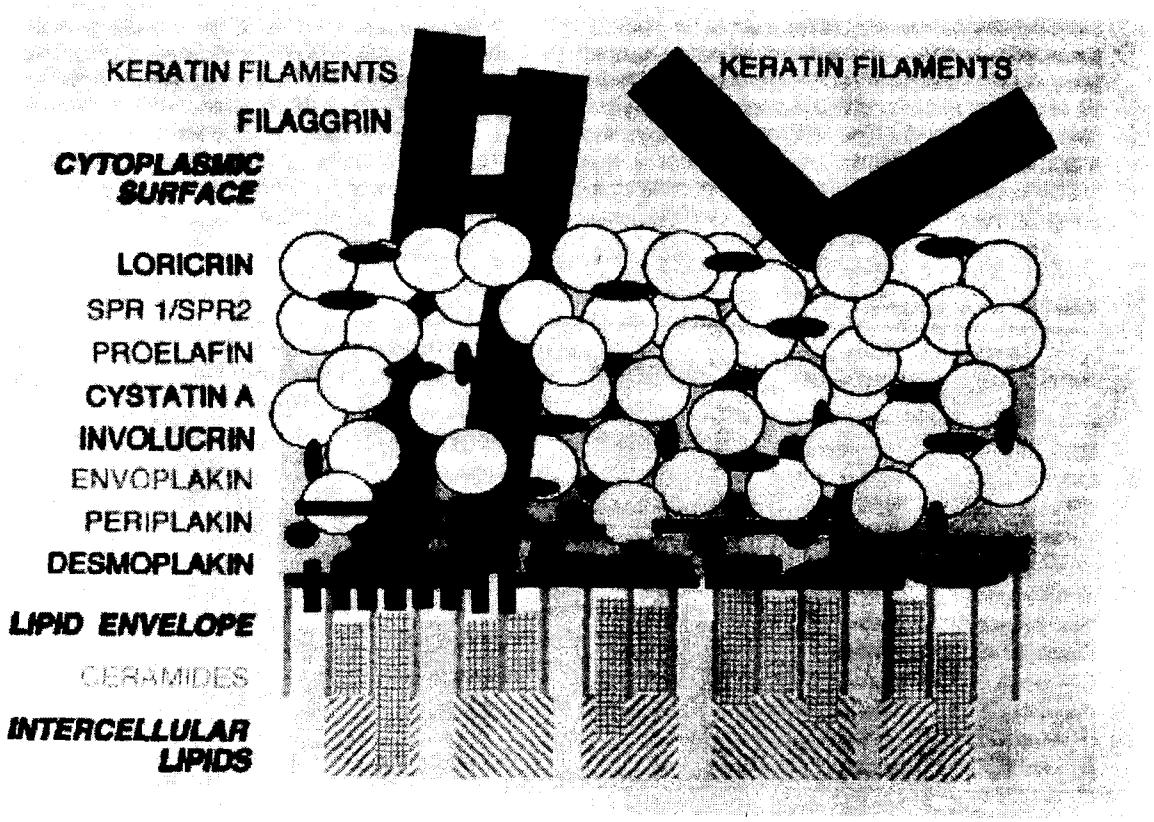
Transglutaminase E (TGase 3)

- 77.8 kD cytoslic zymegoen
- Activated by proteolysis, organic solvent, heat etc.
- Mainly expressed in Stratum Granulosum and functions in Stratum Corneum
- Catalyze crosslinking reactions of numerous cytosolic proteins to loricrin : Spr1,2,3, cystatin, elafin etc.
- Catalytically most active and stable TGase



Summary

Biochemical, enzymatic, immunological, and morphological evidences on ceramide and other lipids lamellae formation, assembly of component proteins of CE and expression and distribution of TGases provided better understanding of CE formation. Pathophysiology of some of the genetic disease is beginning to be clarified. Hope we can utilize the information gained in understanding CE formation would expedite the development of patient treatment processes.


KERATIN FILAMENTS
FILAGGRIN
CYTOPLASMIC SURFACE
LORICRIN
SPR 1/SPR2
PROELAFIN
CYSTATIN A
INVOLUCRIN
ENVOPLAKIN
PERIPLAKIN
DESMOPLAKIN
LIPID ENVELOPE
CERAMIDES
INTERCELLULAR LIPIDS
KERATIN FILAMENTS