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Taming Psoriatic Keratinocytes—PTHs' Uses go up Another Notch

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Abstract The native parathyroid hormone (PTH) and several of its N-terminal adenylyl cyclase-activating fragments and their analogs have become the star stimulators of bone growth for treating osteoporosis, accelerating fracture healing, and strengthening the anchorage of prosthetic bone implants and one of them (Lilly's Forteo™—recombinant hPTH-(1-34) has recently arrived in the clinic. But something entirely different has been lurking in the background—the ability of the adenylyl cyclase stimulating hPTH-(1-34) to calm hyperproliferating keratinocytes and reduce psoriatic lesions. By contrast PTH-(7-34) which cannot stimulate adenylyl cyclase actually stimulates keratinocyte proliferation. Normal keratinocytes make PTHrP after they lift off the basal lamina and have stopped cycling. But they have an unconventional PTH/PTHrP receptorr which is not coupled to adenylyl cyclase. Psoriatic keratinocytes do not make PTHrP and have only a broken-down, proliferation-limiting terminal differentiation-driving Notch–Notch ligand mechanism. Putting these and other facts together produces a possible picture of an exogenously applied adenylyl cyclase-activating PTH pinch hitting for the missing PTHrP and restoring normal keratinocyte proliferative activity epidermal structure by stimulating dermal fibroblasts which do have the conventional adenylyl cyclasse-linked PTHR1 and in response directly or indirectly restore the overlying basal keratinocytes' Notch–Notch ligand terminal differentiation-driving mechanism and consequently a normal epidermal structure. J. Cell. Biochem. 93: 251–256, 2004.

Key words: adenylyl cyclase; bone growth stimulation; Delta 1; dermal fibroblasts; epidermal Ca^{2+} gradient; Jagged 1; keratinocyte differentiation; keratinocyte hyperproliferation; psoriasis; Notch; parathyroid hormone; parathyroid hormone-related protein

PTHs—MULTIPURPOSE BONE-BUILDING TOOLS

Adenylyl cyclase-stimulating parathyroid hormone (PTH) peptides strongly stimulate bone growth in monkeys, rats, and humans and are turning out to be tools for treating osteoporosis, accelerating fracture healing, and strengthening the anchorage of prosthetic implants to bone [Skripitz and Aspenberg, 2000; Whitfield et al., 2000, 2003; Whitfield, 2003, 2004; Andreassen et al., 2004] (Fig. 1). These peptides include the recombinant native human PTH (rhPTH-(1-84), NPS Pharmaceuticals's Preos™) and its N-terminal fragments and their analogs, such as the recombinant rhPTH-(1-34) (Lilly's Forteo™), hPTH-(1-31)NH_2 (Zelos Therapeutics's Ostabolin™), [Lys^{27}]cyclo(Glu^{22}-Lys^{26})hPTH-(1-31)NH_2 (ZelosTherapeutics's Ostabolin-C™) and [Lys^{27}]-cyclo(Glu^{22}-Lys^{26})hPTH-(1-28)NH_2 (Mini-C). Preos™ is nearing the end of its phase III clinical trial, Forteo™ is now available for treating osteoporosis in postmenopausal women and idiopathic osteoporosis in men, and Ostabolin-C™ is in its phase I clinical trial [reviewed in Whitfield, 2004; www.zelostherapeutics.com].

A DIFFERENT USE FOR PTHs—TREATING PSORIASIS

The adenylyl cyclase-stimulating PTHs can do something entirely different. Michael Holick et al. have shown that one of them, hPTH-(1-34), can help the many people suffering from psoriasis and in doing so could tell us a lot about how the N-terminal part of the PTHrP (PTH-related

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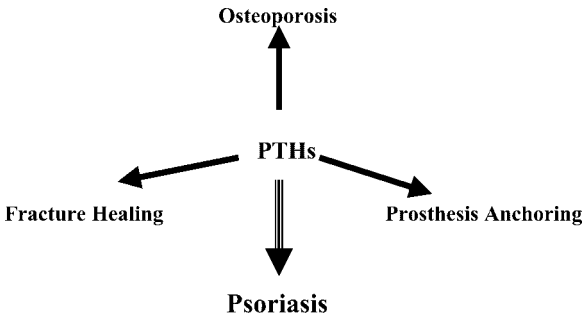

Fig. 1. The different uses for adenylyl cyclase-stimulating parathyroid peptides.

protein) polyprotein controls of the proliferation and differentiation of normal epidermal keratinocytes which make it at a specifc stage in their development from proliferating basal cell to corneocyte cadavers (Fig. 1).

Holick et al. [2003] put hPTH-(1-34) into a percutaneous absorption-enhancing cream—"Novasome A^{TM}"—and then instructed that 0.1 g of this cream containing 20 μg of the peptide be applied twice daily for 2 months to the lesions of persons with chronic plaque psoriasis that had failed to respond to at least one standard treatment. The PTH restored the normal epidermal structure and thus remarkably improved the formerly recalcitrant psoriatic lesions.

Notches, Notch Ligands, PTHrP, and the Normal Epidermis

A normal epidermis is continuously renewed by the proliferation of keratinocytes on the basal lamina. and the stepwise conversion of cells lifting off the basal lamina into stacks of keratin-packed shells—corneocytes [reviewed by Whitfield and Chakravarthy, 2001].

In the basal layer there are clusters of small self-renewing, clonogenic and only intermittently cycling stem cells attached tightly by their β_1 integrins to the basal lamina in niches perched on the crests of the dermal papillae [Lowell et al., 2000; Whitfield and Chakravarthy, 2001] (Figs. 2 and 3). The stem cells are tethered to each other by homodimers of the transmembrane Delta 1 protein [Lowell et al., 2000]. When stem cell progeny are pushed out of the niche the levels of expression of both β_1 integrins and Delta 1 drop and with it the cells' attachment to the basal lamina and each other [Lowell et al., 2000; Whitfield and Chakravarthy, 2001]. At this point the binding of the niche-bound stem cells' Delta 1 to the Notch 1/2

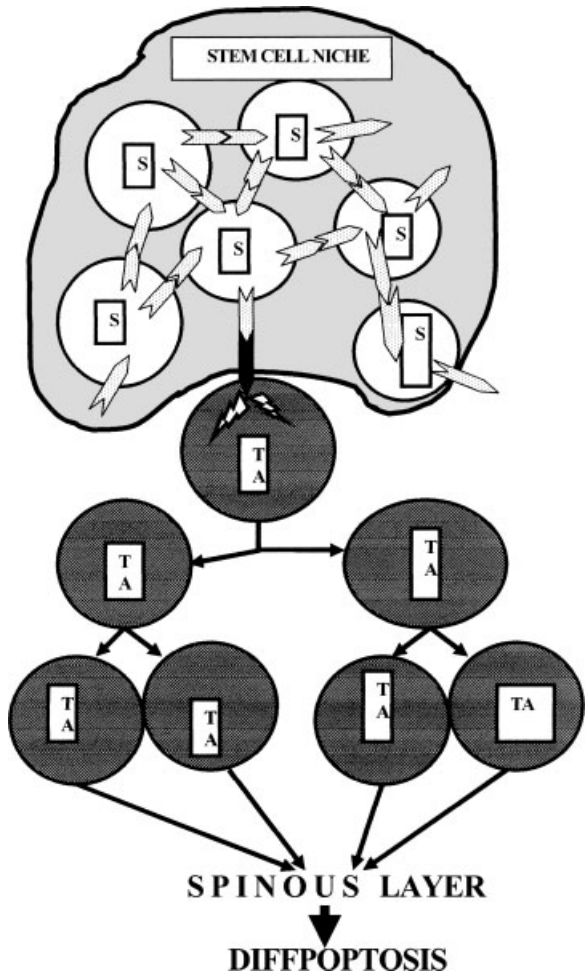

Fig. 2. The intermittently cycling clonogenic cells (S) in the stem cell niche (SCN) on the epidermal basal lamina are tethered together by homodimers of Delta 1 [Lowell et al., 2000]. However, when a cell is pushed out of the niche its Delta 1 level falls and it upregulates the expression of other ligands such as Jagged 1 [Nickoloff et al., 2002]. Binding and the activation of its Notch receptor (➽)triggers the conversion of the evicted cell into a transit amplifying (TA) cell with only a limited cycling potential and a commitment to terminal differentiation. The basal TA cell can generate about 32 progeny which lift off the basal lamina,become spinous cells and thus start the differentiation/apoptosis-like process that Whitfield and Chakravarthy [2001]have called ''diffpoptosis''that ends in keratin-packed corneocytes.

receptors on the evicted cells starts the differentiation process [Lowell et al., 2000; Rangarajan et al., 2001; Okuyama et al., 2004]. The cells also express another Notch ligand, Jagged 1, which increases in the suprabasal cells up to the granular layer while the expression of Delta 1 falls off [Nickoloff et al., 2002]. The Notch signaling causes the departing cells to become larger, faster-cycling TA ("transit amplifier") cells with cell cycle genes stimulated by

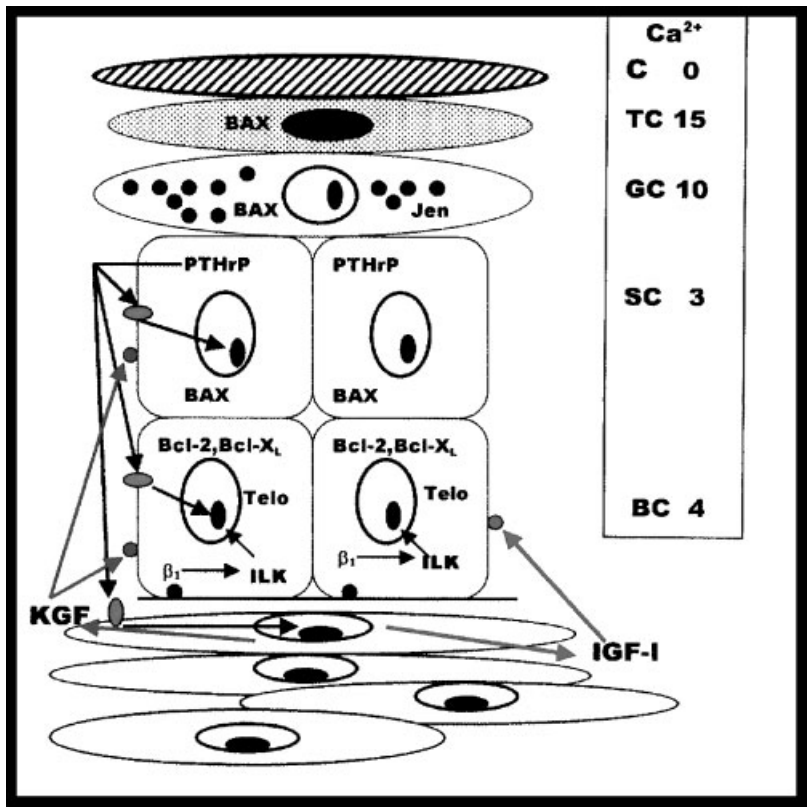

Fig. 3. The skin with its transepidermal Ca^{2+} gradient (the values are from human skin and expressed as mmole/Kg dry weight), PHTrP expression and busy commerce between keratinocytes and dermal fibroblasts. Each TA cell that has been pushed out of the stem cell niche in Figure 2 needs a low external Ca^{2+} concentration and signals from basal lamina-attached β_1 integrins to proliferate. The integrin signals stimulate ILK kinase which in turn prevents the destruction of cytoplasmic β-catenin, which can then reach the nucleus and stimulate the expression of cell cycle genes and suppress the expression of the proliferation-suppressing E-cadherin. Both the stem cells and their TA cell progeny in the basal cell (BC) layer protect themselves from apoptotic suicide by making Bcl-2 or Bcl-X_L proteins and suppressing their Bax killer protein. They also express telomerase (Telo) which prevents their chromosomes' telomeres from shortening with each of the occasional round of stem cell replication ant the approximately 5 rounds of TA cell replication. As the cycling TA cells are pushed further away from the stem cell niche, the level of basal lamina-tethering and β_1 integrin signaling drops. Along with this drop, ILK activity fades and β-catenin is increasingly destroyed before reaching the nucleus. At a critical distance from the niche the cell cycle counter reaches 0. There is then a Ca^{2+} surge in the cell which in collaboration with Notch signaling blocks the cell from starting another cell cycle by, among other things, stimulating the expression of the cycle-suppressing $p21^{CIP1/WAF1}$ protein [Nickoloff et al., 2002; Nicolas et al., 2003]. The cell lifts off the basal lamina into the spinous cell (SC) layer. The new spinous cell stops expressing the anti-apoptosis Bcl-2 and Bcl-X_L proteins as well as Telo and starts making the Bax killer protein in preparation for its ultimate ''diffpoptotic'' demise in the upper layers. The spinous cell also starts making full-length PTHrP and chops it into fragments which have different receptors (⬬) with different functions [Whitfield et al., 1996]. The keratinocytes have no conventional PTHR1 receptors, but the N-terminal fragments diffuse down and activate the conventional PTHR1 receptor on the dermal fibroblasts (⬮) the signals from which trigger the expression of factors such as KGF (keratinocyte growth factor) and IGF-I (insulin-like growth factor-I) which communicate across the basal lamina with the keratinocytes. This possible interaction at the basal lamina between a Notch ligand such as Jagged 1 on, or from, dermal fibroblasts and the TA keratinocytes' Notch3 receptors concentrated at the basal laminar level [Nickoloff et al., 2002] drives the TA cell's cycle shutdown and the TA cell ⇒ spinous cell transition when the cell's internal Ca^{2+}level surges and the β_1 integrins and their signaling have fallen below a critical density. The final stages of differentiation begin when the cell is pushed up into the granular layer (GC) and are driven by signals from CaRs (Ca^{2+} receptors) activated by a sharp rise in the external Ca^{2+} concentration and specific transcription factors such as the ets-family Jen protein [Andreoli et al., 1997; Chattopadhyay and Brown, 2003]. At the head of the Ca^{2+} gradient at the top of the cell stack the CaR signaling in the transitional cells (TC) triggers the apoptosis-like mechanism that destroys the cellular organelles, expels the accumulated Ca^{2+} and converts the cell into one of the hardened keratin-packed shells of the stratum corneum. A far more detailed and referenced description of this journey from stem cell to corneocyte shell can be found in Whitfield and Chakravarthy [2001].

β-catenin which is saved from destruction by ILK kinase activated by signals from β_1 integrins but also with cell cycle counters, which, when they reach 0 (32 progeny), tell the cells to turn off their cell cycle genes, dismantle their cell cycle machinery, change the parts of their Notch–Notch ligands maturation engine, stop making the proliferation-compatible keratins 5 and 14, start making keratins 1 and 10, lift off the basal lamina, and become spinous cells [Whitfield et al., 1995; Lowell et al., 2000; Whitfield and Chakravarthy, 2001; Nickoloff et al., 2002] (Figs. 2 and 3).

The basal stem cells do not express a PTH/PTHrP receptor, but TA keratinocytes do start making and deploying a PTH/PTHrP receptor [Errazahi et al., 1998] (Fig. 3). However, this receptor responds to PTH and PTHrP differently from the conventional PTHR1 receptor—normal keratinocytes do not express their gene for the conventional PTHR1 although some might do so during "immortalization" [Henderson et al., 1992; Hanafin et al., 1995; Orloff et al., 1995; Sharpe et al., 1998] And this receptor, whatever it may be, does not couple to adenylyl cyclase, although the keratinocytes have the machinery to do so because they can respond to the β-adrenergic receptor-activating isoproterenol with a large burst of adenylyl cyclase activity [Whitfield et al., 1992; Orloff et al., 1995]. Moreover, keratinocytes can be made to respond to PTH-(1-34) with a burst of adenylyl cyclase activity if they are engineered to express the conventional PTHR1 receptor [Orloff et al., 1995] However, the TA keratinocytes do not start making the PTHrP to stimulate these unconventional PTH receptors until they have stopped proliferating and lifted off the basal lamina [Juhlin et al., 1992; Danks et al., 1995; reviewed by Whitfield and Chakravarthy, 2001] (Fig. 3). Besides being an autocrine/paracrine/I ntracrine stimulator of various activities in both the basal and suprabasal keratinocyes (Fig. 3), its level is likely an indicator of the size of the suprabasal cell population. The amount of PTHrP coming down from the suprabasal cells could tell the basal cells with their unconventional PTH receptors and the dermal fibroblasts, with their conventional adenylyl cyclase-coupled PTHR1 receptors [Hanafin et al., 1995; Pun and Tam, 1995; Maioli et al., 2002] how many suprabasal cells there are and to adjust their proliferation accordingly.

The Psoriatic Keratinocytes' Missing Delta, Jagged, Notches, and PTHrP

The beefy red psoriatic patch or plaque is characterized by hyperproliferating keratinocytes that can invade the normally forbidden suprabasal layers and a stack of corneocytes which are reluctant to desquamate. This keratinocyte hyperproliferation is triggered locally by bacterially or otherwise activated Langerhans cells and driven by a torrent of cytokines and other factors including NO that causes the local dermal blood vessels to dilate and give the patch its redness [Nickoloff et al., 2000; Lebwohl, 2003].

Contributing to the hyperproliferation is a local breakdown of the transepidermal Ca^{2+} gradient that normally controls proliferation and terminal differentiation [Menon and Elias, 1991; reviewed in Whitfield and Chakravarthy, 2001] (Fig. 3). The psoriatic keratinocytes also express Notch/Notch ligands either weakly or not at all which means the failure of the main maturation engine [Thélu et al., 2002]. And this is could well be the reason for the hyperproliferation because Nicolas et al. [2003] have shown that eliminating Notch 1 in mice causes epidermal hyperplasia through a proliferogenic upregulation of β-catenin and Gli2 expression. And along with these striking differences they don't make PTHrP [Juhlin et al., 1992], probably because of the breakdown of the Notch/Notch ligands mechanism that would normally have restrained β-catenin and Gli2 expression and driven the stem cell ⇒ TA cell transition, with the resulting stratification, generation of the Ca^{2+}gradient and PTHrP expression in the spinous and granular layers [Juhlin et al., 1992; Danks et al., 1995; Lowell et al., 2000; Whitfield and Chakravarthy, 2001; Thélu et al., 2002].

HOW MIGHT PTH's TAME PSORIATIC KERATINOCYTES?

The adenylyl cyclase-stimulating hPTH-(1-34) can stop keratinocyte hyperproliferation and restore normal keratinocyte stratification in humans and both it and PTHrP (1-34) reduce keratinocyte proliferation in mouse epidermis, a model for testing anti-psoriasis drugs [Holick et al., 1994]. On the other hand, bPTH-(7-34), which stimulates PKC (protein kinase C) but not adenylyl cyclase [Jouishomme et al., 1992; reviewed in Whitfield, 2004] competes with hPTH-(1-34) for the conventional PTHR1 recep-

tor and actually stimulates keratinocyte proliferation and hair growth in mice [Holick et al., 1994; Schilli et al., 1997]. This argues strongly for the targets of topically applied, psoriasis-suppressing hPTH-(1-34) not being keratinocytes but some other cells with conventional PTHR1 receptors.

It seems likely that hPTH-(1-34) stands in for the psoriatic keratinocytes' missing PTHrP and, through cyclic AMP made by PTHR1-activated adenylyl cyclase, directly or indirectly restores the differentiation-driving Notch/Notch ligand engine that has broken down in psoriatic keratinocytes [Thélu et al., 2002]. An example of hPTH-(1-34) stimulating a Notch/Notch ligand mechanism has been reported by Calvi et al. [2003]. They found that this PTH expanded the HSC (hematopoietic stem cell) pool in mouse bone marrow by stimulating osteoblastic cells lining the trabecular or endocortical bone surfaces to express large amounts of the Jagged 1 Notch ligand which probably attached to and triggered the proteolylic processing/activation of Notch receptors on the surfaces of cells in adjacent HSC niches [Zhu and Emerson, 2004].

Since hPTH-(1-34) cannot persuade keratinocytes to make the cyclic AMP needed to reduce keratinocyte proliferation, where are the target cells with adenylyl cyclase-coupled PTHR1 receptors? The most obvious candidates are the dermal fibroblasts lying directly beneath the basal lamina which are constantly conversing with the keratinocytes via various factors [Hanafin et al., 1995; Pun and Tam, 1995; Maioli et al., 2002] (Fig. 3). This would mean that the hPTH-(1-34) from Holick et al's "Novasome A^{TM}" cream reaches the dermis where it activates the fibroblasts' conventional PTH receptors, the signals from which induce the cells to produce factors such as KGF and IGF-I and to directly or indirectly rebuild the Delta/Jagged-Notch engine in the overlying keratinocytes which drives their normal proliferation and stratification with generation of a normal transepidermal Ca^{2+} gradient and resumption of PTHrP expression (Fig. 3).

SUMMARY AND FUTURE PROSPECTS

In conclusion, exogenous hPTH-(1-34)'s ability to tame hyperproliferating psoriatic keratinocytes and restore a normal epidermis points to the importance of the N-terminal part of endogenous PTHrP from suprabasal keratinocytes and the cross-talk it drives between keratinocytes and dermal fibroblasts in the maintenance of normal skin structure. Apparently a normal epidermis requires either endogenous PTHrP or a N-terminal PTH "pinch-hitter" and an intact Notch/Notch ligand maturation-driving engine. Does a PTHrP- or hPTH-(1-34)—triggered burst of adenylyl cyclase activity induce dermal fibroblasts to express large amounts of Jagged 1, like Calvi et al. [2003] hPTH-(1-34)-stimulated mouse bone marrow osteoblastic cells, that can contact the Notch receptors on the basal keratinocytes and modulate their proliferation? Perhaps significantly, Nickoloff et al. [2002] have found that a particular Notch, Notch 3, is strikingly concentrated where basal keratinocytes and dermal fibroblasts meet—the basal lamina. Or do the dermal fibroblasts make a paracrine factor(s) that upregulates the keratinocytes' Notch mechanism to maintain normal keratinocyte proliferation and differentiaition and PTHrP production? In fact this factor could be Jagged 1 because Nickoloff et al. [2002] have shown that soluble Jagged 1 can trigger maturation and stratification of submerged human keratinocyte monolayers. Clearly finding the answers to these questions about PTH/PTHrP and dermal fibroblast-keratinocyte interactions may lead to the treatment of other skin conditions, including baldness [Holick et al., 1994; Schilli et al., 1997], that involve dysregulated keratinocyte proliferation.

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