Role of CPI-17 in restoring skin homoeostasis in cutaneous field of cancerization: effects of topical application of a film-forming medical device containing photolyase and UV filters

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Abstract: Cutaneous field of cancerization (CFC) is caused in part by the carcinogenic effect of the cyclobutane pyrimidine dimers CPD and 6-4 photoproducts (6-4PPs). Photoreactivation is carried out by photolyases which specifically recognize and repair both photoproducts. The study evaluates the molecular effects of topical application of a film-forming medical device containing photolyase and UV filters on the precancerous field in AK from seven patients. Skin improvement after treatment was confirmed in all patients by histopathological and molecular assessment. A gene set analysis showed that skin recovery was associated with biological processes involved in tissue homoeostasis and cell maintenance. The CFC response was associated with over-expression of the CPI-17 gene, and a dependence on the initial expression level was observed (P = 0.001). Low CPI-17 levels were directly associated with pro-inflammatory genes such as TNF (P = 0.012) and IL-1B (P = 0.07). Our results suggest a role for CPI-17 in restoring skin homoeostasis in CFC lesions.

Key words: actinic keratoses – CPI-17 – cutaneous field of cancerization – expression array – PPP1R14A

Accepted for publication 20 May 2013
assumption after Eryf-AK treatment, the subjects were classified as
fast responders (FR) versus slow-partial responders (PR) (Table S1).

The differential gene expression analysis of CFC pretreatment
versus posttreatment assessment failed to detect deregulated genes
after correction for multiple testing. However, over-expressions of
CPI-17 (2-fold increase, P = 0.039) and WDR72 gene
(1.9-fold increase, P = 0.040) were detected in FR subgroup.
CPI-17 expression differences between FR and PR subgroups were
confirmed by RT-PCR (P = 0.001) (Figure 1a). In contrast, no
significant differences (P = 0.211) were observed for WDR72
(Figure 1b). Initial CPI-17 levels were higher in FR than PR patients
(P = 0.045; Figure 1a).

Pathological conditions such as inflammation modulate CPI-17
expression (10). Thus, expression of TNF and IL-1B cytokines were
evaluated. FR showed lower expression levels of TNF (P = 0.012)
and IL-1B (P = 0.07) after Eryf-AK (Figure 1c,d). Posttreatment PR
lesions still showed a high IL-1B expression level (P = 0.038).

Gene set analysis identified 150 biological functions (P < 0.005;
Table S2) associated with CFC, which were classified in four major
biological clusters: ‘generation of reactive oxygen species’, ‘mech-
anism involved in DNA repair’, ‘cell division process’ and ‘lipids’. Sixty
six processes were associated with Eryf-AK treatment
(P < 0.005, Table S3), which were grouped as ‘cell communication
and signalling’, ‘cell adhesion’ and ‘tissue development’.

Gene set analysis according to treatment response identified 24
GOs over-expressed in FR subgroup mostly involved in cell
response and homeostasis and 28 GOs’s associated with PR
lesions which were related to inflammation and cytokine produc-
tion, apoptosis and lipid metabolic processes (Table 1).

Conclusions
We identified biological processes associated with CFC such as
ROS production and DNA damage repair processes that may be
induced in part by CPDs and/or lipid metabolism. Lipid content
changes are important in AK and BCC (11). After treatment, we
found over-expression of fundamental processes related to tissue
reconstitution (cell communication, signalling and adhesion).

Based on treatment response, treated PR biopsies showed an
over-expression of apoptotic process, lipid metabolism, cytokine
production and inflammation which are directly related to AK.
Inflammation is important for AK maintenance which is abolished
by the topical use of diclofenac combined with hyaluronic acid
through a selective inhibition of COX2 (12–14). The histopatho-
logical evaluation showed the presence of AK in at least 20% of
the biopsy specimen from PR patients. In treated FR subgroup,
we observed an improvement in cell homeostasis and adhesion

Table 1. Gene ontology terms detected by gene set analysis in posttreatment biopsies by response subgroup

| Response to treatment | Gene Ontology terms (ID and P-value) |
|-----------------------|--------------------------------------|
| Fast responders (FR)  | Cell-cell signalling (007267, P = 3.02E-06); Embryonic development (009790, P = 1.15E-05); Cell development (0048468, P = 1.46E-05); Secretion (0046903, P = 2.10E-05); Regulation of secretion (0051046, P = 1.22E-04); Muscle contraction (0006936, P = 1.87E-04); Chemical homeostasis (0048878, P = 2.44E-04); Anterior/posterior pattern formation (0009952, P = 3.05E-04); Cellular homeostasis (0019725, P = 4.93E-04); Embryonic development ending in birth or egg hatching (0009792, P = 6.73E-04); Chordate embryonic development (0043009, P = 1.8E-03); Homoeostatic process (0042592, P = 1.30E-03); Ion homeostasis (0050801, P = 1.53E-03); Secretion by cell (0032940, P = 1.53E-03); Response to steroid hormone stimulus (0048545, P = 2.00E-03); Cell fate commitment (0045165, P = 2.19E-03); Response to hypoxy (0001666, P = 2.22E-03); Cellular chemical homeostasis (0050822, P = 2.23E-03); Regulation of blood vessel size (0050880, P = 2.76E-03); Smooth muscle contraction (0006939, P = 3.26E-03); Response to inorganic substance (000135, P = 4.09E-03); Response to hormone stimulus (009725, P = 4.37E-03); Neurological system process (0050877, P = 3.08E-02); Cell morphogenesis (0003920, P = 1.17E-00); Organic acid metabolic process (0006082, P = 3.35E-18); Carboxylic acid metabolic process (0019752, P = 3.35E-18); Lipid metabolic process (0006629, P = 1.81E-13); Cellular lipid metabolic process (0044255, P = 8.13E-13); Lipid biosynthesis process (000610, P = 2.09E-09); Regulation of immune response (0050776, P = 1.09E-08); Steroid metabolic process (0008202, P = 7.70E-08); Regulation of interleukin-6 production (0032675, P = 2.07E-07); Positive regulation of cytokine production (0001817, P = 2.07E-07); Positive regulation of immune response (0050778, P = 2.81E-06); Carbohydrate metabolic process (0005975, P = 3.67E-05); Leucocyte migration (0050900, P = 4.62E-05); Neutral lipid metabolic process (0006638, P = 1.65E-04); Glyceral ether metabolic process (0006662, P = 1.65E-04); Regulation of lipid metabolic process (0019216, P = 1.80E-04); Positive regulation of cytokine production (0001819, P = 1.83E-04); Response to drug (0042493, P = 3.12E-04); cellular carbohydrate metabolic process (0044262, P = 3.61E-04); locomotory behaviour (0007626, P = 3.91E-04); Lipid storage (0019915, P = 8.60E-04); Response to organic substance (0001033, P = 2.62E-03); interleukin-12 production (0032615, P = 2.95E-03); Apoptosis (0006915, P = 2.96E-03); Negative regulation of cytokine production (0001818, P = 3.01E-03); Programmed cell death (0012501, P = 3.68E-03); Response to molecule of bacterial origin (002237, P = 3.96E-03); Regulation of programmed cell death (0043067, P = 4.06E-03); Amine metabolic process (009308, P = 4.74E-03). |

1 Patients were classified as fast responders and partial responders based on the histopathology assessment after Eryf-AK treatment (see Table S1).
2 The ID numbers and the P-values are indicated for each GO.
which correlates with the improvement in histopathological measures (Puig et al. 2012; submitted for publication).

**CPI-17** over-expression was associated with normal phenotype recovery. *CPI-17* is one of the major Ser/Thr phosphatase isoforms, and its activation suppresses the MYPT1-PP1δ activity resulting in muscle contraction (10). *CIP-17* expression is detected in multiple cell types (15–17) involved in several processes (16,18). MYPT1 inhibition results in more prominent focal adhesions and absence of cell migration (19). *CPI-17* is directly associated with focal adhesion kinase (20) and located at focal adhesions in fibroblasts and keratinocytes (21). MYPT1-PP1δ complex can also regulate the dephosphorylation of retinoblastoma protein (pRb) (22) which shows a deregulated activation in AK. We observed that inflammation modulates *CPI-17* expression in CFC. Thus, processes such as DNA damage or ROS production may cause *CPI-17* down-deregulation, which could lead to uncontrolled MYPT1-PP1δ activity. Deregulated phosphatase activity in CFC may affect cell motility, cell adhesion and cell cycle control mediated by pRb.

In conclusion, 1-month Eryf-AK treatment improved the field of cancerization and restored normal phenotype in at least a subset of samples, through *CPI-17* up-regulation.

**Acknowledgements**

S.P and J.M designed the research study. S.P collected skin biopsies and obtained the clinical data. J.A.P.B performed the whole-genome expression arrays. M.P performed the RT-PCR. J.A.P.B, F.G.G and J.D analysed and interpreted the results; J.A.P.B wrote the paper. C.T reviewed the manuscript. The present project was partially funded by a grant from ISDIN. The research at the Melanoma Unit in Barcelona is partially funded by Grants from Fondo de Investigaciones Sanitarias (09/01393), Spain; by the CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain; by the AGAUR 2009 SGR 1337 of the Catalan Government, Spain. This work is also partly supported by grants BIO2008-04212 from the Spanish Ministry of Science and Innovation (MICINN) and PROMETEO/2010/001 from the GVA-FEDER.

**Conflict of interests**

The authors state no conflict of interests. The sponsors had no role in the design and conduct of the study and interpretation of data.

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