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Role of p63 and related pathways in epithelial stem cell proliferation and differentiation and in rare EEC-related syndromes

Epistem

Integrated Project
Life Sciences, genomics and biotechnology for Health; Priority 1

Final Publishable Executive Summary

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Start date of project: 01/01/2006 Duration: 48 months

Coordinator: Peter Vandenabeele
Flanders Interuniversity Institute for Biotechnology

Publishable executive summary

Project summary

The focus and expected results of the Epistem consortium are, to better understand the molecular basis of Ectodermal dysplasia disease, to generate new knowledge on genes involved in epidermal development and disease and to translate this knowledge into applications that enhance human health. To this end both fundamental and applied research is involved. Epistem integrates multidisciplinary and coordinated efforts to understand the molecular basis of factors involved in epidermal stem cell generation, maintenance and differentiation and skin disease. Moreover, the core molecule that will be studied in this IP is p63 (and related pathways), a molecule genetically proven to be involved in the development of rare skin diseases such as EEC syndrome (Ectrodactyly-ectodermal dysplasia–clefting), Hay-Wells (AEC) syndrome, Limb-mammary syndrome, ADULT syndrome, Rapp-Hodgkin syndrome and non-syndromic split hand-split foot malformation. Collectively, the prevalence of ectodermal dysplasia syndromes (EDS) is estimated at 7 cases in 10,000 births. Currently there is no cure for these patients. By creating the Epistem consortium we want to address from different angles (genetics, gene profiling, molecular and cellular biology, structural biology, drug design, bioinformatics) the molecular pathways involved in epidermal dysplasia syndromes making use of different technologies (mutation analysis, micro-array, ChiP, transgenes, proteomics, *in vitro* skin cultures, crystallography, etc). Our consortium brings together leading European clinicians, geneticists, molecular and cellular biologists, structural biologists, a drug designer and bioinformatics specialists in the field of p63 (and related molecules) research. From 2007, two additional partners from 'third countries' joined the consortium. One is a specialist in rare rare craniofacial anomalies and will help in the collecting and characterizing p63 mutant patients and will concentrate on the enamel hypoplasia phenotype, which is almost a consistent feature of EEC syndromes. The second will study the mechanistic details of the alternative promoter use for the balanced expression of p63 isoforms.

Project objectives

To summarize, this project has the following main objectives:

- Collecting and culturing of keratinocytes from EDS patients with p63 mutations and extensive analysis of phenotype-genotype correlation. These keratinocytes will be manipulated to uncover the role of p63 proteins and pathways in normal and abnormal skin development.
- Building relevant *in vitro* and *in vivo* skin disease models for studying the role of p63 in EDS disease and the genetic assessment of novel pathways discovered during this project. These models will also be used for drug assessment.
- Provide insight into the regulation and involvement of p63 and related pathways in skin differentiation, the maintenance of the proliferative capacity of epithelial stem cells and the transition of ectodermal cells to epidermal stem cells.
- Screening for and design of novel therapeutic drugs, based on three dimensional p63 models, which will refold/reactivate or inhibit p63 mutants and induce biological responses in relevant disease models.
- Create a bioinformatics platform with graphical interface that integrates the separate data sets obtained throughout the project (vertical comparative genomics) with public

datasets from other species and the phylogenetic analysis of the p63, p53 and p73 protein family (horizontal comparative genomics) to predict p63, p53 and p73 functional interaction partners.

Contractors involved

Participant . Role*	Participant Number	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	Peter Vandenabeele	VIB/UGent	Belgium	1	48
CR	2a 2b	Hans van Bokhoven CMBI	RUNMC	The Netherlands	1	48
CR	3a 3b	Gerry Melino Eleanora Candi	UTV-DEMBS	Italy	1	48
CR	4	Gian Paolo Dotto	UNIL	Switzerland	1	48
CR	5	John McGrath	KCL	United Kingdom	1	48
CR	6	Klas Wiman	KI	Sweden	1	48
CR	7	Volker Doetsch	UNIFRA	Germany	1	48
CR	8	Roberto Mantovani	UniMI	Italy	1	48
CR	9	Istituto Oncologico del Mediterraneo SpA	I.O.M.	Italy	1	48
CR	10	Daniel Aberdam	INSERM	France	1	48
CR	11	GeneSpin	GeneSpin	Italy	1	48
CR	12	Piranit Kantaputra	CMU	Thailand	13	48
CR	13	Jingde Zhu	SJTU	China	13	48

*CO = Coordinator; CR = Contractor

Coordinator contact details

Prof. Dr **Peter Vandenabeele**, Flanders Institute for Biotechnology, Ghent, Belgium, has been appointed by the Consortium as Coordinator (peter.vandenabeele@dmbr.ugent.be).

The Task Manager, Ir. **Jelle Verspurten** (jelle.verspurten@dmbr.ugent.be), who is hired on the project, supports the coordinator in the administrative tasks.

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Work performed and achieved results

The epidermis is a multilayered, stratified epithelium that provides a physical barrier for the organism, protecting it from dehydration UV radiation and a variety of environmental insults. The epidermis is continuously renewed due to a regulated balance between proliferation and differentiation. This renewal capacity is due to the presence of epidermal stem cells. The concept that the transcription factor p63 is essential for formation of the epidermis arises from two lines of evidence. First, mice lacking p63 have no epidermis, and they also lack epithelial appendages, such as mammary, salivary and lachrymal glands, hair follicles and teeth. Second, mutations in the human p63 gene are responsible for ectodermal dysplastic syndromes. But how does p63 contribute to the formation of the epidermis and maintenance of epithelial stemness? Do the p63 patient mutations have any diagnostic or prognostic value? What are the underlying mechanisms of its functions? What is the molecular pathogenesis of ectodermal dysplasia syndromes? Can we design a cure to 'reactivate' mutant p63 molecules?

We studied the p63-dependent signaling pathways to understand the role of p63 in ectodermal dysplasia syndromes, making use of different technologies (mutation analysis, micro-array, ChiP, transgenes, proteomics, *in vitro* skin cultures, crystallography, etc.). In addition, studies within our consortium indicated the involvement op the p63 transcription factor in the maintenance of epithelial stemness.

Overall, EPISTEM forms a tight network with many interactions between the different work packages and partners involved. This enabled us to accomplish substantial breakthroughs in our understanding of the role of p63 in health and disease. The major achievements to date are:

- Establishment of the largest collection in the world of biopsies, cells, and DNA derived from ~300 patients with mutations in the p63 gene.
- We are currently gaining profound knowledge on the genotype-phenotype correlation in ectodermal dysplasia patients. This can be of major social impact because it may become possible to predict disease progression or spontaneous disease improvement, depending on the type of mutation found in these patients.
- We established cellular systems, both human and mouse, that mimic embryonal stem cell progression to ectodermal commitment and keratinocyte differentiation. Moreover, we (as well as other groups) found that p63 is crucial for maintaining the

epidermal stemness. These insights could have significant importance for skin regeneration therapies such as in burn patients.

- Continuous and overlapping expression profiling enabled us to get a better view of which target genes of the p63 transcription factor are important for driving epidermal differentiation and maintaining the stemness character of the epidermal stem cells. We developed BioIT approaches that enable comparison of the different data sets obtained within the consortium and available in literature.
- We developed (and are further developing) a number of mouse models by introducing human patient-related p63 mutations in the mouse, which could mimic human disease. This will enable us to better understand the effect of the different p63 mutations in patients and to test new theratpeutic approached developed within the Epistem consortium.
- Finally yet importantly, we identified small compounds that can rescue the transcriptional deficiency of some patient-related p63 mutants *in vitro*. We are currently screening the effects of these drugs in several cellular assays available within the consortium. The ultimate goal is to test these compounds on the mouse ectodermal dysplasia disease models that are being developed within the EPISTEM consortium.

In summary, we believe that this EU integrated project brought together and strengthened the European research community focusing on the role of the p63 as a major transcription factor involved in epithelial stemness, development, and disease.

Glossary

EDS:

The ectodermal dysplasia syndromes (EDS) are a group of inherited autosomal dominant human diseases. The disorders are congenital, diffuse, and nonprogressive. Ectodermal dysplasia manifests as the abnormal development or growth of tissues and structures that are developed from the outer embryonal layer, ectoderm. In this condition, there is usually abnormal development of skin, hair, teeth, nails and several exocrine glands, such as sweat and sebaceous glands. Morbidity and mortality is related to the absence or presence of eccrine and mucous glands.

EEC:

EEC Syndrome (Ectrodactyly Ectodermal dysplasia) is a rare form of ectodermal dysplasia inherited as an autosomal dominant genetic trait, the symptoms of which can vary from mild to severe. The most common symptoms found in patients with EEC Syndrome are missing or irregular fingers and/or toes (ectrodactyly), abnormalities of the hair and glands, cleft lip and/or palate, unusual facial features, or abnormalities of the eyes and urinary tract.

p63:

p63 is a transcription factor that belongs to the p53 family of tumor suppressors. p63 expression is crucial for the development of stratified epithelial tissues such as epidermis, breast and prostate and is responsible for maintaining the proliferative potential of the epithelial stem cells (= stemness). The gene for p63, TP63, encodes multiple protein isoforms that possess both transactivating and transcriptional repressor activities. These proteins regulate a wide spectrum of target genes. p63 is also implicated in tumor formation and progression in stratified epithelia.

Plan for using and disseminating the knowledge

During the course of the project (4 years), the consortium members gave about 104 talks at international conferences or institutes. Next to the talks, at least 55 additional abstracts were presented at international conferences. The work resulted in 97 peer reviewed a1 publications in international journals.

In 2007, we were allocated a session dedicated on p63 at the Zurich ESDR meeting (European Society of Dermatological Research). Three Epistem members gave an invited lecture.

The Epistem Ghent 2008 conference was held in Ghent, Belgium from February 27th to February 29th 2008. 110 registered participants from 17 countries attended this first mini-symposium.

The Epistem – CDD p63/p73 Workshop 2009 was held in Toronto, Canada from August 31th to September 2nd 2009. It was a joined effort of Cell Death & Differentiation Conferences and Epistem and was held at the Ben Sadowski Auditorium at Mt. Sinai Hospital. There were 113 registered participants from 18 countries and 38 speakers. Registration to the meeting was handeled by our project manager Jelle Verspurten and was directed via the Epistem website. The website of the Epistem consortium has been kept up-to-date and our visibility was significantly extended by organizing the Ghent 2008 mini-symposium and the p63/p73 workshop. In addition, all partners clearly mentioned the existence of the EU-funded Epistem consortium in their many talks at international conferences.

For the full PUDK we refer to the end of the document.

Additional information

Project website: http://www.epistem.eu

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Project Logo: