Review

UHN Microarray Centre

Getting under your skin – using keratinocytes to identify p63 gene targets

Review of Feature Article: Vigano MA, Lamartine J, Testoni B, Merico D, Alotto D, Castagnoli C, Robert A, Candi E, Melino G, Gidrol X, Mantovani R. New p63 targets in keratinocytes identified by a genome-wide approach. EMBO J, 2006, 25(21):5105-16

Now that the human genome has been sequenced, the task of identifying the function of each gene has begun. For transcription factors, the identification of specific targets will allow us to better understand complex transcriptional networks and to treat diseases associated with mutations in transcription factor genes.

p63 is a master regulatory gene of multilayered epithelia such as keratinocytes[1] and there is evidence that p63 is involved in developmental processes[2,3]. In particular, the ΔNp63α isoform is thought to play a key role in the asymmetric division of epithelial cells and is involved in ankyloblepheron-ectodermal defects (AEC), ectrodactily, ectodermal dysplasia (EEC), and the split hand/foot malformation (SHFM) syndromes[4]. One of the difficulties in finding targets specific to p63 lies in its homology with other transcription factors, like p53.

Conventional strategies for finding genes targeted *in vivo* by p63 include profiling analysis of cells in which p63 and p63α are overexpressed, silencing p63 with siRNA, and by studying p53-controlled genes as p63 is homologous to p53. More recently, however, the ChIP-on-chip technique, which combines chromatin immunoprecipitation (ChIP) and the use of DNA microarrays (chips) containing CpG islands and promoter regions, has been used to identify genome-wide transcription factor targets.

Vigano *et al*. used the ChIP-on-chip technique to screen for p63 targets using both promoter arrays and human CpG island 12k arrays. HaCaT keratinocytes were used as p63 is involved in the development of ectodermal tissues and HaCaT cells partially mimic basal keratinocytes. By identifying novel targets of p63, scientists could better understand the molecular mechanisms of this complex transcription factor.

This study found that many of the p63 targets are themselves transcription factors or co-regulators. As expected, p63 regulated classes of genes important for developmental and differentiation processes like cell adhesion, cytoskeleton, cell proliferation, and cell cycle. Unexpectedly, p63 was found to target genes involved with immunity and vascular processes. Other novel targets included 8 genes carrying the WD40 domain, which is involved in signal transduction, pre-mRNA processing, and cytoskeleton assembly[5], genes acting as MAPK modulators and genes belonging to the ubiquitin-dependent protein processing pathways. Vigano *et al*. speculate that ubiquitin-dependent regulation targets may themselves be regulators of p63 activity in a way similar to the p53/MDM2 feedback regulatory loop.

The targets were validated by promoter transactivation studies, immunostaining normal tissue to confirm regulation by p63, and by expression analysis in differentiated HaCaT cells and in cells overexpressing ΔNp63α, which verified ΔNp63α targets were regulated during keratinocyte differentiation. *In vivo* p63 binding experiments in primary keratinocytes were also performed and results show that half of the loci were also bound by p53, which indicates that both proteins regulate common sets of genes. One of the limitations of the ChIP-on-chip technique identified in this study was the varying degree of gene enrichment during the generation of ChIP probe amplicons. The authors suggest that variations could have been a result of sub-optimal selection of the amplified region or due to binding of p63 mediated by other DNA-binding proteins. In addition, ChIP experiments represent average binding within the entire cell population that is heterogeneous with respect to cell-cycle phases and thus the *in vivo* affinity of p63 for specific targets may have varied. Another source of

variation that is not discussed is the way in which immunoprecipitated DNA was amplified (by LM-PCR). Such issues should be kept in mind when performing ChIP-on-chip studies.

This study illustrates the robustness of the ChIP-on-chip approach. Vigano *et al.* were able to identify 183 new ΔNp63α target genes, 88% of which were effectively bound in vivo, thus enabling us to better understand its molecular mechanisms. This study has also demonstrated that ChIP-on-chip is a relatively accurate high-throughput method for identifying transcription factor targets.

References:

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