Researcher Experience Sharing: Application of shRNA

Title: **Krüppel-Like Factor 4 (KLF4) suppresses neuroblastoma cell growth and determines non-tumorigenic lineage differentiation**

Application of shRNA

Speaker: **Dr Elly Ngan, Assistant Professor, Department of Surgery, HKU**

Abstract: Neuroblastoma (NB) is an embryonal tumor and possesses a unique propensity to exhibit either a spontaneous regression or an unrestrained growth. However, the underlying mechanism for this paradoxical clinical outcome still remains largely unclear. Quantitative RT-PCR analysis on 102 primary NB tumors revealed that lower *KLF4* expression is frequently found in the unfavorable NB (Mann-Whitney test, p=0.027). In particular with the high-risk factors such as age of patient >1 year, *MYCN* amplification and low *TRKA* expression, the decreased expression of *KLF4* was significantly associated with an unfavorable NB outcome. Despite knock-down of KLF4 alone is not sufficient to increase tumorigenicity of NB cells *in vivo*, stable expression of *KLF4* short hairpin RNA in Be(2)-C cells significantly promoted growth of NB cells and inhibited cell differentiation toward fibromuscular lineage. In concordant with these observations, overexpression of KLF4 in SH-SY-5Y cells profoundly suppressed cell proliferation by direct up-regulation of cell-cycle inhibitor protein $p21^{WAF1/CIP1}$, and knocking down $p21^{WAF1/CIP1}$ could partially rescue the suppressive effect of KLF4. Importantly, KLF4 overexpressing cells have lost their neuroblastic phenotypes, they were epithelial-like, strongly substrate-adherent, expressing smooth muscle marker and became non-tumorigenic, suggesting that KLF4 expression is crucial for lineage determination of NB cells, probably, favoring spontaneous tumor regression. Subsequent global gene expression profiling further revealed that transforming growth factor beta (TGFβ) and cell cycle pathways are highly dysregulated upon KLF4 overexpression, and myogenic modulators, *MEF2A* and *MYOD1* were found significantly upregulated. Taken together, we have demonstrated that KLF4 contributes to the favorable disease outcome by directly mediating the growth and lineage determination of NB cells.

Technology Seminar

Title: **Improved exon capture reagents for focused medical re-sequencing**

Speaker: **Dr Mark Behlke, Chief Scientific Officer, Integrated DNA Technologies**

Abstract: "Next generation" sequencing methods are emerging as the next frontier in medical diagnostics. For example, DNA sequencing can be used to find actionable mutations in tumors to help direct therapeutic decisions. Exon-capture methods are useful to reduce sample complexity, lowering cost and improving throughput. The entire human exome can be captured using millions of low-quality oligonucleotides made on microarrays. Complexity can be further reduced by using custom sets of capture probes specific for a small number of genes (typically 5-200). For these smaller sets, high quality oligonucleotides made using standard synthesis methods can be used. IDT's "xGen Lockdown™ Probes" are 5'-biotin labeled 120mer DNA oligonucleotides made in ultra-small scale. These reagents are far higher quality than array oligos and meet quality control requirements for medical diagnostics. Use of these reagents for medical re-sequencing by Foundation Medicine and the Washington University Genome center will be discussed.

About our speaker:

Mark A. Behlke, M.D., Ph.D.

Chief Scientific Officer

Integrated DNA Technologies, Inc.

As the Chief Scientific Officer, Dr. Behlke directs activities at IDT across a variety of new product development and basic research areas including functional genomics (RNAi and antisense), DNA thermodynamics, probe chemistries, amplification methods, and next generation sequencing technologies. He has directed research activities at IDT since joining the company in 1995. Dr. Behlke (together with Dr. John Rossi, from the Beckman Research Institute at the City of Hope) is a scientific co-founder of Dicerna Pharmaceuticals, located in Boston, Massachusetts and is a member of the Dicerna Scientific Advisory Board.

Before joining IDT, Dr. Behlke was a Physician Postdoctoral Fellow of the Howard Hughes Medical Institute at the Whitehead Institute for Biomedical Research, MIT, where he studied human sex determination in the laboratory of Dr. David Page. He was a Resident Physician in Internal Medicine and Fellow in Endocrinology at Brigham and Women's Hospital, Boston. Dr. Behlke received his M.D. and Ph.D. degrees from Washington University, St. Louis in 1988, where he studied immunogenetics in the laboratory of Dr. Dennis Loh. He received his B.S. degree from the Massachusetts Institute of Technology in 1981.

Mark A. Behlke is an inventor on over 20 issued US patents, has numerous pending patent applications, and is author of over 80 scientific publications in peer-reviewed journals. He is an internationally recognized expert in nucleic acid technologies.