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Concept Clearance

Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) Program

Introduction

NIEHS has been at the forefront of epigenetics research at the NIH and is now primed to further address the role of the environment in disease susceptibility as a function of changes to the epigenome. To date, this Institute has made great strides in identifying global methylation patterns/marks as a result of exposure to a diverse array of toxicants and exposures. These marks tell only part of the story, however, as they remain largely descriptive - essentially as a biologic readout of a far more complex regulatory process. While this line of investigation represents an important first step in understanding how exposures could contribute to disease, in order to fully understand the underlying mechanisms, efforts must now move more towards revealing how these exposures are impacting/interacting with the functional/regulatory elements that lead to correlative pattern changes in methylation. To address this critical gap in knowledge, we propose development of the Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) Program. TaRGET represents an unprecedented opportunity to establish a potentially cross-divisional activity that would take advantage of:

- infrastructure (e.g., rigorously tested antibodies, data quality metrics, sample processing pipelines) established by ENCODE program and the NIH Roadmap Reference Epigenome Mapping Consortium (REMC);
- high throughput, next generation sequencing technologies and computational data analysis tools to comprehensively evaluate the response to exposure;
- collaborative expertise and involvement of DIR, DNTP and DERT scientists and the extramural community.

The first phase of TaRGET (TaRGET I) will be a research component featuring R01 projects. In September 2010, the NAEHS Council approved a Concept Clearance for a PAR entitled, “Environmental Influences on Transcriptional Regulation”. The first release of this PAR is scheduled for late 2012 and the NIEHS anticipates making 3-4 awards per year. TaRGET I will solicit applications that will examine a broad range of

transcriptional activities and the influence of toxicants on these processes. Applications will be encouraged that elucidate the role of toxicants on chromatin remodeling, transcription factor binding sites, *cis* regulatory modules (CRMs), ncRNA functions, nucleosome positioning and other aspects of chromatin biology and functional genomic elements, addressing how toxicants ultimately influence transcription and/or gene expression profiles in model systems.

The second phase of this program (TaRGET II) will establish a production component to tackle a fundamental concern by validating the robustness/feasibility of using peripheral blood lymphocytes (PBLs) as a proxy for changes in epigenetic marks in target tissues using rodent models.

The third phase (TaRGET III) will support the expansion of data from exposed cohorts that are currently collecting global DNA methylation profiles and/or RNA microarray data. Investigators will be supported to generate additional data related to transcription factors binding sites, chromatin accessibility, post translational histone modifications and RNA polymerase II complex data.

The fourth phase, or TaRGET IV, will support integrated analyses in population-based studies, making use of a number of genomic and epigenomic databases to develop more comprehensive epigenomics/genomics analyses e.g., by overlay of epigenomic marks mapping data with GWAS SNP or haplotype blocks that have previously been associated with increased disease risk.

Research Goals and Scope

TaRGET I: Environmental Influences on Transcriptional Regulation PAR

There is a clearly emerging need for research that examines the impact of the environment on transcriptional processes, which may in turn alter or reprogram a cell's epigenome. TaRGET I will support research activities that consider various aspects of transcriptional regulation, encompassing epigenetic processes, chromatin dynamics, e.g., higher order structure/chromatin states, nucleosome positioning, regulatory genomic elements, transcription elongation, and ncRNA functional changes following environmental exposures. A variety of model systems may be used for *in vivo* and *in vitro* studies. Toxicants proposed should have disease relevance as well as evidence of inducing disrupted epigenetic processes and/or reprogramming. GWAS data may be used to inform these proposals with regard to identifying potential environmental stressor targets in regulatory regions of the genome.

This phase will support applications that probe transcriptional units and regulatory domains that acquire mutations and alter or manifest potential disease relevant changes in epigenetic programming as a consequence of environmental influence. Additionally, it is important to consider transcriptional units and regulatory domains that could acquire mutations and consequently alter changes in epigenetic programming which only manifest as a function of stressor response. The converse must also be considered,

i.e., epigenetic changes/methylation status may possibly influence genome stability, leading to increased mutability. Functional readouts will be required from applicants and may include but are not limited to: alterations in gene expression profiles; changes in mutability of genome; changes in higher order chromatin dynamics, e.g., chromatin looping and may be associated with changes in *cis* and *trans* regulation of gene expression.

Applications submitted in response to TaRGET I should select chemicals that are associated with phenotypic outcomes in cells and/or tissues and where there is preliminary evidence e.g., heavy metals and endocrine disrupting chemicals (EDCs). Selection of toxicants used for exposure studies should be done based on evidence of specific pathway perturbations, e.g., DNA repair or receptor mediated pathways.

TaRGET I will stimulate research in this exciting area incorporating an exposure paradigm by solicitation via a program announcement (PAR) which affords review by a Special Emphasis Panel (SEP) and provides three opportunities for applicants to submit applications. Moreover, applications submitted in the second and third year receipts may be able to benefit from analyses of data from TaRGET II and III. The R01 mechanism will be used.

TaRGET II: Resource Generation

This goal of this component of TaRGET is to develop a community resource that examines epigenomic marks (DNA methylation, histone modifications) and functional genomic elements (transcriptional units and regulatory domains) in disease rodent models following challenge with an environmental toxicant, identifies any changes in peripheral tissues (blood) and compares those with changes observed in other target tissues .

NIEHS funds a number of investigators who are collecting blood/peripheral blood lymphocytes or other easily accessible, peripheral cell types from participants in large cohort studies, in an effort to assess the effects of exposure on human populations. Many of these investigators are beginning to investigate whether exposure-induced epigenetic changes underlie the disease observed in these populations. Knowing that epigenetic profiles vary significantly from tissue to tissue prompts the following question: Are PBLs (or other surrogate cell types) an appropriate tissue in which to identify exposure-induced epigenetic changes that occur in tissues more directly related to the disease of interest (e.g., dopamine neurons and Parkinson's disease)?

This question has been addressed to some level within the Roadmap Epigenomic Program. The Mapping Centers have performed extensive epigenomic mapping of multiple normal cell types, and some investigators from the Roadmap Epigenomics of Human Health and Disease RFA have made comparisons of profiles in blood and diseased tissue in affected individuals. TaRGET II will incorporate exposure into this paradigm, and moreover address the key question of whether epigenetic/transcriptional changes induced by exposure are conserved across cell types using environmentally

relevant exposures in a rodent disease model. At initial stages of these awards, investigators will be required to: identify which chemicals will be used; establish a range of doses to ascertain what levels of exposure correlate to epigenomic and genomic changes; and establish a schedule of dosing at different times throughout development and aging to identify windows of susceptibility. Additionally, susceptible and resistant strains could be used to address questions regarding susceptibility based on genetic background.

TARGET II would begin with a small pilot study(s) to provide proof of principle needed to support expansion of the project. This pilot study(s) would focus on one representative toxicant from each of several broad classes, such as: heavy metals, endocrine disrupting chemicals, and particulates, and would investigate a limited number of epigenetic/transcriptional features, most likely DNA methylation, selected histone modifications, and a transcription factor binding site. This pilot study could runparallel with the first release of the TaRGET I PAR on transcription described above.

TaRGET II will provide a critically important resource to the ES community of investigators who are currently pursuing research in epigenetics within the context of exposure and disease pathogenesis. The expected outcomes of this initiative are: 1) identification of common and divergent epigenetic changes induced by a variety of exposures under a variety of conditions and 2) determination of whether epigenetic changes induced by exposure are conserved across tissues. Although the specific epigenetic changes observed in mice may not translate directly to human studies, the data obtained from this study will serve as proof of principle that cross-tissue comparisons are appropriate.

Utilizing cooperative agreements (U01) or a contract mechanism, TaRGET II will support projects that assess the constellation of changes in epigenomic marks (methylation, histone modifications) and functional genomic elements (genes, transcripts, and transcriptional regulatory regions) occurring after exposure to a defined panel of disease relevant environmental toxicants, in a targeted set of tissues, including peripheral cell types such as lymphocytes. Applications will be solicited by an RFA to support U01s (cooperative agreement) or an RFP to support contracts for this production effort.

TARGET III: Population Based Studies-developing more comprehensive datasets

An increasing number of studies are beginning to conduct integrative analyses of epiegenomic/genomic datasets from individuals affected with a range of diseases, e.g., Chron's disease, diabetes, SLE. While GWAS has had some success in identifying sequence variation linked to specific diseases, the integration of GWAS data with epigenomic data represents a more powerful approach. TaRGET III is a second population based component of the program. The NIEHS is currently supporting several population based studies where investigators are generating data on global methylation patterns that are influenced by an exposure, most often with data collected from PBLs. This component of TaRGET would supplement these existing research grants to

support the generation and integration of more comprehensive datasets that include additional epigenomic marks and functional elements; The expanded datasets may include data on PTHMs, ncRNAs, chromatin accessibility and/or transcription factor binding sites. Studies that will involve use of peripheral tissues to assess changes in marks will be informed by data generated in TaRGET II. The competitive supplement mechanism will be used to support the development of more comprehensive datasets from exposed cohorts.

TARGET IV: Population Based Studies-integrative analyses

TaRGET IV proposes to conduct integrated analyses of genomics, epigenomics, and other extensive data sets (e.g. derived from TaRGET III, EWAS, Exposome, GWAS, ENCODE, REMC, IHEC, GENEVA, etc.) to allow a more comprehensive understanding of the complex interactions of genes, genomic elements, epigenomics and environment for human disease outcomes. CNVs and other structural mutations influencing genomic instability as well as EGP gene variants related to DNA repair, oxidative stress, and cell cycle, division, and signaling, and other known environmentally-responsive elements could also be explored in this component.

This phase will allow the mechanisms of higher transcriptional regulation and epigenomics processing (beyond just methylation readouts) to be integrated with existing genomic and environmental data, and could utilize recently developed GWAS x E analytical methods. An example that illustrates the utility of this kind of integrative analysis is recent data suggesting that a large majority of SNPs from GWAS studies are located in regulatory regions and are likely affecting transcriptional regulation through environmental exposures and epigenomic mechanisms This final component will therefore, likely lead to new mechanistic paradigms of how environmental exposures impact a complex array of human diseases.

Applications would require collaboration between investigators with environmental exposure expertise and investigators with expertise in bioinformatics, with an emphasis on epigenomic and genomic elements analysis. These analyses could inform R01 applications submitted to the Phase I PAR in subsequent submissions (2nd and 3rd year receipts. Due to the strong focus on integrative analyses, a substantial portion of the budget of these supplements would support bioinformatics capabilities TaRGET IV awards will use R21s to support this activity.