CBI STUDENT SABBATICAL PROPOSAL
July 6, 2012

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2009 Cohort

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Sabbatical Duration: 10 weeks

Time Frame: February 2013- April 2013

Proposal Summary: Together, we will investigate the phenotypic and specific cell fate consequences of inhibiting the p300/CH1 domain on NANOG expression in cancer cells. Possible connections between the CH1 domain and NANOG will be established through a combination of functional and *in vivo* assays. The data produced will directly inform and advance the developing model of my thesis research.

Background

Modern cancer biology is increasingly focused on the intrinsic heterogeneity within cancer cell populations. Not all neoplastic cells possess the capacity to initiate or support long-term tumor growth, despite the supposed clonal nature of the cancer. This has led to the recognition of cellular hierarchies in cancer that resemble the organization found in stem cell systems– the so-called 'cancer stem cell (CSC) hypothesis' (Reya T, *et al*. 2001; Dalerba P, *et al*. 2007). Recently, the embryonic self-renewal factor NANOG has been reported to be required for tumor development and acquisition of CSC-like properties (Jeter CR, *et al.* 2009; Jeter CR, *et al*. 2011; Zbinden M, *et al*. 2010; Chen C, *et al*. 2011), suggesting that NANOG may be an attractive target for eliminating the CSC populations. As a transcription factor, though, NANOG is not a tractable drug target, and very little is known about the protein structure overall. Thus, other methods for inhibiting NANOG and related 'undruggable' transcription factor targets are sorely needed. In principle, one way to inhibit NANOG would be to target the coactivators that regulate transcription of the NANOG gene itself.

The transcriptional coactivators CBP/p300 are large, multidomain histone acetyltransferases (HATs) involved in initiating gene transcription and expression (Fig. 1A). Once localized to the gene promoter, the HAT domain of CBP/p300 can modify histone tails and facilitate chromatin relaxation, while other domains (*e.g.* CH1, KIX, IBiD) can recruit additional coactivators and lead to the complete assembly of the transcriptional preinitiation complex. The modular nature of these domains confers considerable functional flexibility, allowing them to interact with >400 different protein partners and regulate a diverse number of transcriptional programs including embryogenesis, metabolism, and cell growth (Goodman RH & Smolik S. 2000). The utilization of the various CBP/p300 domains can vary greatly depending on gene context. For example, the activator CREB recruits CBP/p300 via the KIX domain and requires HAT activity to turn on CREB-target gene expression (Korzus E, *et al*. 1998). In contrast, nuclear receptor-induced transcription requires the NRID domain, but not the HAT (Korzus E, *et al*. 1998). Unfortunately, despite its central role in regulating many essential and pathological pathways, the specific CBP/p300 functions are largely undefined in most contexts. Thus, while CBP/p300 may be attractive targets for therapeutic intervention, the development of new small molecule modulators have been hindered by a lack of detailed information about the relevant targets within CBP/p300.

Studies in embryonic stem cells have indicated a close correlation between p300 and NANOG activity (Chen X, *et al*. 2008; Zhong & Jin. 2009), suggesting that targeting p300 could potentially affect NANOG and NANOG-driven tumorigenesis. So far, though, little is known about the role(s) of CBP/p300 in promoting NANOG activity or self-renewal in human cancers. My research seeks to dissect and pharmacologically target the relevant coactivator functions in regulating NANOG expression in cancer cells.

Preliminary Work

Based on the p300/NANOG connection in ESCs, we initially hypothesized that NANOG expression in human cancer cells would be p300-dependent. After depleting p300 levels by RNAi, we observed dramatic reductions NANOG transcript levels in two different cancer cell lines (Fig. 1B), indicating that p300 is required for NANOG expression. Furthermore, our collaborator Prof. Quintin Pan at OSU, has seen that p300 knockdown

inhibited NANOG-dependent phenotypes such as tumorsphere formation (Fig. 1C). Together, these results confirmed that p300 is necessary for NANOG expression.

Next we sought to identify the crucial coactivator functions for NANOG expression. The most salient feature of CBP/p300 as coactivators is the HAT activity. Therefore, we tested the effects of overexpressing wild-type (WT) and catalytically inactive HAT mutants ($HAT^{WY/AS}$) on a *NANOG* promoter-driven reporter. Both WT and $HAT^{WY/AS}$ overexpression dose-dependently enhanced promoter activity, and unexpectedly, HAT inactivation had no inhibitory effect on reporter enhancement relative to WT (Fig. 1D), suggesting that the HAT activity may be dispensable. Therefore, some other domain(s) within CBP/p300 must provide the essential coactivator functions for NANOG expression.

To further define these essential domains, we generated in-frame deletions of the various CBP/p300 domains and tested their effects relative to WT in the same reporter assay. Deletion of the CH1 domain (ΔCH1) had the most significant effect on CBP/p300 activity, attenuating reporter enhancement by >60% (Fig. 1E). Significantly, introduction of a single amino acid substitution (C379A) known to disrupt folding of the CH1 domain (Newton AL, *et al*. 2000) recapitulated the effect of deleting the entire domain (Fig. 1E). Collectively, these results strongly suggest that the CH1 domain is necessary for NANOG expression and function in cancer cells.

From a small molecule perspective, the CH1 domain is a challenging target due to its large binding interface and lack of a defined ligand-binding pocket (Fig. 1F)(Freedman SJ, *et al*. 2002; Dames SA, *et al*. 2002). Despite these obstacles, the natural product chetomin and hydrogen bond-surrogate peptide mimics have been shown to successfully disrupt activator-CH1 domain interactions and inhibit CH1-dependent gene activation under hypoxic conditions (Kung AL, *et al*. 2004; Henchey LK, *et al*. 2010). So far these compounds have only been used in the context of hypoxia, and work is currently underway investigating their potential applications in NANOG regulation.

Proposed Sabbatical

Much of the work done at Michigan has been devoted to identifying the relevant domains of CBP/p300, often in an artificial, *in vitro* system like a reporter assay. These experiments facilitate manipulation and modification of the coactivators– which was useful for initially identifying the CH1 domain– but do not necessarily address the endogenous effects of the CH1 domain on NANOG expression. Furthermore, *in vitro* experiments do not reveal any details about changes in cell fate, such as if a NANOG-positive CSC has been forced to differentiate. These questions are of particular importance to our developing model of CH1/NANOG-mediated tumorigenesis.

Our laboratory here lacks the knowledge and access to these types of phenotypic experiments, leaving a potentially unmet need for ways to directly translate the *in vitro* findings described above into more biological contexts. Fortunately, however, we have a well-established collaboration with Prof. Quintin Pan, a specialist in head and neck oncology at OSU, whose laboratory has the necessary resources to conduct *in vivo* and CSC-based experiments. Therefore, my proposed research sabbatical entails working on-site with Prof. Pan's group characterize the CH1/NANOG connection and relevance at the cellular level.

During the course of the sabbatical, I will learn a variety of phenotypic assays (*e.g.* tumorsphere, mouse xenograft) that can then be used to explore the effects of the CH1 domain on NANOG regulation. For instance, NANOG knockdown has been shown to greatly reduce tumorsphere formation in head and neck cancer cells (*Quintin Pan, manuscript submitted*). If NANOG expression is dependent upon the CH1 domain, then expressing a dominant-negative form of the CH1 domain will not only reduce NANOG expression in head and neck cancer cells, but also inhibit tumorsphere formation. Preliminary data suggests this is, in fact, the case. Additionally, administration of CH1-targeting inhibitors like chetomin should also abrogate tumorsphere formation, confirming that the CH1 domain is a potential target for malignant, NANOG-dependent phenotypes.

Since NANOG expression enriches for CSCs (Jeter CR, *et al*. 2011; Chen C, *et al*. 2011; Zbinden M, *et al*. 2010), my data may further suggest that CH1/NANOG modulation could affect the CSC population. While at OSU, I will explore this connection by testing the effects of CH1-targeting inhibitors on the expression of putative CSC markers like ALDH1. Changes in the fraction of ALDH1-positive cells can indicate effects on the CSC pool. Furthermore, apoptotic and cell cycle analyses will reveal the ultimate cell fate as a result of CH1/NANOG inhibition. When combined, these results should allow us to propose a model that encompasses both molecular-level requirements for oncogene activation and their cellular-level impacts. This model can then be used to guide further studies into aspects of specific protein-protein interactions involving the CH1 domain, as well as the larger role of specific coactivators in self-renewal transcriptional programs.

Sabbatical Impact on Thesis Research

The proposed sabbatical with Prof. Pan will advance my thesis research in two significant ways: 1) The results from the phenotypic and CSC assays will directly contribute to the emerging model of CH1-dependent NANOG regulation and tumorigenesis. These experiments are logical extensions of my current data and provide an excellent example of research transitioning from a more chemically based, *in vitro* system to a more biological one. 2) The methods learned will be generally applicable in future experiments. Cellular and *in vivo* validation of proposed mechanisms are frequently required in biological research, and the techniques learned during the sabbatical will add to the repertoire of experiments I am capable of performing.

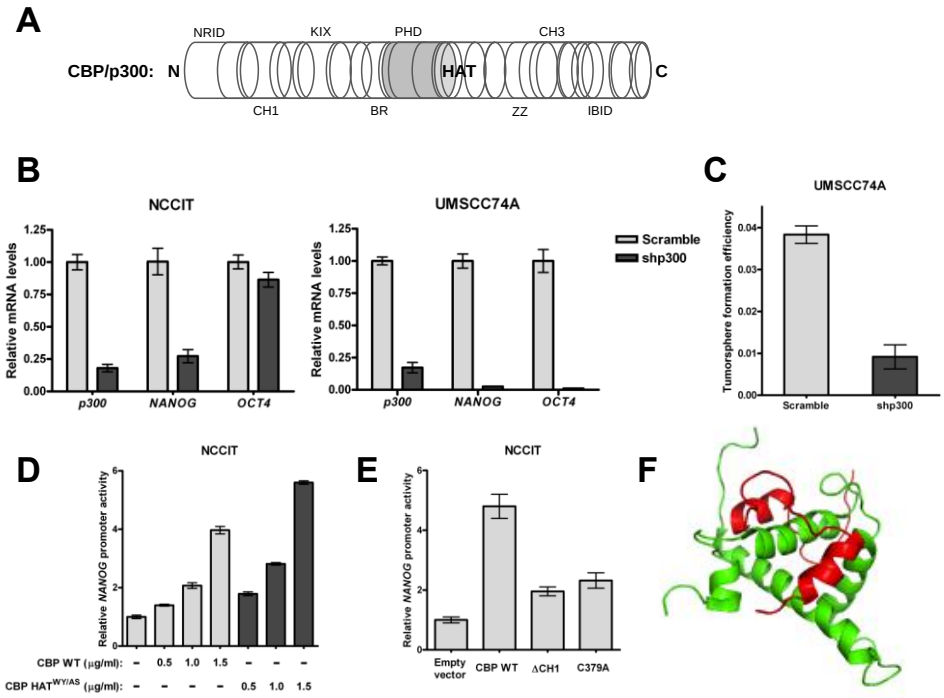

Figure 1 | The CBP/p300 CH1 domain is necessary for NANOG expression in cancer cells. A) Schematic layout of CBP/p300. Major functional domains are indicated. B) Knockdown of p300 downregulates *NANOG* mRNA levels in both pluripotent embryonal carcinoma cells (NCCIT) and head and neck squamous cell carcinoma cells (UMSCC74A). C) p300 knockdown reduces UMSCC74A tumorsphere formation. D) CBP WT and $HAT^{WY/AS}$ enhance *NANOG* promoter activity in NCCIT cells. Concentrations of transfected plasmids are indicated. E) Disruption of the CH1 domain attenuates CBP-mediated reporter enhancement. F) Solution structure of CBP CH1 domain (*green*) bound by the transcriptional activation domain of HIF-1α (*red*)(PDB: 1L8C).

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