Research Projects:

II. 藉由轉錄因子JDP2 研究Wnt 訊息傳遞與染色質調節來進行小腦顆粒性細胞在腫瘤生成的探討--Study of Wnt signaling and chromatin regulation by transcription factor JDP2 in oncogenesis of cerebellar granule cells. (2012~2015) NSC project

細胞內外的訊息傳遞與反應調節，使真核細胞的細胞週期經由增生、分化、凋亡和老化等時間點的轉變程序，而形成新的細胞品系，此類時間點稱爲不可回溯點，一般來說是由遺傳密碼與表觀遺傳（epigenetic）來決定，在此我們著重於研究染色質調控所造成的基因表現來瞭解細胞進入不可回溯點的機制。先前的研究已鑑別出基因轉錄因子JDP2（Jun dimerization protein）屬於AP-1（activator protein 1）家族中的一員，結果指出JDP2 爲AP-1 阻遏物，能在控制氧化壓力（oxidative stress）的狀況下，引起細胞生長、細胞分化與複製老化，另外藉由DNA 結合區與組蛋白乙醯化作用抑制區（the domain of inhibition of histone acetylation；INHAT）之研究來歸納出JDP2 具有多種功能。在遏制細胞週期中，JDP2 也扮演關鍵性的角色，主要調控cyclin A2 、cycling E2 與p16Ink4a 的表現，而上述因子爲p19Arf-Mdm2-p53-p21-cyclin/cycling-dependent kinase（CDK）與p16Ink4a-cycling/CDK-Rb-E2F 所形成之網狀調節系統的成員。然而JDP2 對於p53-p21 與Rb-E2F 基因在細胞週期分子層面的調節作用仍尚待闡明。我們近期的研究成果指出Wnt訊息傳遞途徑相關基因，如: 淋巴增強子結合蛋白1(Lymphoid enhancer binding protein；LEF1)、Wnt1 誘導型訊息傳遞途徑蛋白2（Wnt 1 inducible signaling pathway protein 2；Wisp2）與分泌型捲曲相關蛋白2（secreted frizzled-realated sequence protein 2；Sfrp2）等，爲JDP2 的目標基因。但JDP2 如何調控Wnt 訊息傳遞，以及在p53-p21 與Rb-E2F細胞週期相關途徑中，Wnt 如何傳遞基因訊息等問題，仍然需要解決。因此研究主要目標爲闡述JDP2 在細胞週期調節與染色質調控上，對於抗腫瘤生成過程中所扮演的角色。爲了評估JDP2 在癌症治療上所具有的潛力，我們會著手研究JDP2 在正常小腦顆粒性細胞（granule cells）與贅生的神經管胚細胞瘤（neoplastic medulloblastoma）中的表現。同時也會測定JDP2 在神經管胚細胞瘤中的腫瘤抑制效能，並評估JDP2 及其衍生物在抑制腫瘤生成上，以誘發細胞老化與遏制細胞週期來作爲癌症治療藥物是否可行。

研究的主要具體目標爲：

1. 檢測在細胞週期調控與**Wnt** 訊息傳遞過程中，**JDP2** 所扮演的角色研究JDP2 基因如何調控表觀遺傳與轉錄作用，在p16Ink4a-cyclin/CDK-Rb-E2F 與p19Arf-Mdm2-p53-p21-cyclin/CDK 網絡構造下，經由Wnt 訊息傳遞途徑，進行探討JDP2 所引發細胞週期的抑制作用。

2. 研究在複製老化過程中，**JDP2** 扮演的角色探討經由氧氣誘發JDP2 基因表現的機制，並藉由JDP2 誘導p16Ink4a 與p19Arf 基因表現，而誘發複製老化作用產生。

3. **JDP2** 蛋白質的結構分析在組蛋白修飾、細胞核運輸、DNA 結合、二聚體群落、核小體集合與轉錄抑制作用的過程中，來鑑別JDP2 關鍵性的氨基酸和不可缺少的分子區域。

4. 探討在抗腫瘤生成過程中，**JDP2** 扮演的角色使用JDP2-/-p53-/-雙基因剔除小鼠來檢測JDP2 的活性與其衍生物對於治療腫瘤的潛力，並應用此動物模式來培養神經管胚細胞瘤，並定義JDP2 在抑制腫瘤生成過程中所扮演的角色。

In response to internal and external signals, eukaryotic cells undergo changes to enter a new cell lineage through the alteration of programs for **proliferation, differentiation, apoptosis** and **senescence** at certain points in the cell cycle. Such a point is designated as a "**point of no return,**" and, in general, is determined by genetic and epigenetic codes. Here, we focus on **the chromatin regulation of gene expression** to understand the mechanism by which cells commit to this fate or "**point of no return.**" We have identified the transcription factor, Jun dimerization protein 2 (JDP2), a member of the AP-1 family. We have found that JDP2 is an AP-1 repressor that controls cell growth, cell differentiation and replicative senescence induced by oxidative stress. These multiple functions of JDP2 are determined by the DNA-binding domain and the domain of inhibition of histone acetylation (INHAT). Moreover, JDP2 plays a critical role in cell cycle arrest through regulated expression of cyclin A2, cyclin E2 and p16Ink4a, which are involved with the p19Arf-Mdm2-p53-p21-cyclin/cyclin-dependent kinase (CDK) and p16Ink4a-cyclin/CDK-Rb-E2F networks. However, the molecular basis for JDP2-mediated cell cycle regulation in p53-p21 and Rb-E2F remain to be elucidated. Recently, we have identified the Wnt-signal-related genes such as the genes encoding LEF1 (lymphoid enhancer binding protein), Wisp2 (Wnt 1 inducible signaling pathway protein 2) and Sfrp2 (secreted frizzled-related sequence protein 2) as the targets of JDP2. However, how JDP2 controls the signaling of Wnt and how Wnt transmits signals to the cell cycle pathways of p53-p21 and Rb-E2F, remain to be solved. Thus, this proposal is aimed at exploring the role of JDP2 in cell cycle regulation and chromatin regulation to commit anti-oncogenesis. To evaluate the potential role of JDP2 in cancer therapy, we will investigate the expression of JDP2 in normal cerebellum granule cells and its neoplastic medulloblastoma. We will also examine the tumor suppressor function of JDP2 in medulloblastoma and assess the role of JDP2 as a therapeutic reagent to induce cellular senescence and cell cycle arrest for anti-oncogenesis.

1. Investigation of the role of JDP2 in cell cycle control and Wnt signaling

We will conduct a study of the transcriptional and epigenetic control of JDP2 in networks of p16Ink4a-cyclin/CDK-Rb-E2F and p19Arf-Mdm2-p53-p21-cyclin/CDK to induce the cell cycle arrest through Wnt signaling.

2. Investigation of the role of JDP2 in replicative senescence

We will conduct a study on the mechanisms underlying the oxygen-induced expression of JDP2 and the induction of p16Ink4a and p19Arf by JDP2 to induce replicative senescence.

3. Structural analysis of the JDP2 protein

We will identify the domains and critical amino acids of JDP2 required for histone modification, nuclear transport, DNA binding, dimer formation, nucleosome assembly and transcriptional repression.

4. Investigation of the role of JDP2 in anti-tumorigenicity

We will identify the role of the tumor suppressor activity of JDP2 using JDP2-/-p53-/- double knockout mice as well as determine the potential therapeutic activity of JDP2, and prepare a mouse model of medulloblastoma and the trafficking.

Key words: cell cycle control, Wnt signal, replicative senescence, JDP2, histone chaperone, tumor suppressor