• 計畫中文名稱	核基質結合區域蛋白 SATB2 在調控骨母細胞存活與代謝上可能扮演的角色研究		
• 計畫英文名稱	Roles of Nuclear Matrix-Attachment Region Protein SATB2 in Regulation of Osteoblast Survival and Metabolism		
• 系統編號	PC9609-3887	• 研究性質	基礎研究
• 計畫編號	NSC96-2628-B038-005-MY3	• 研究方式	學術補助
• 主管機關	行政院國家科學委員會	• 研究期間	9608 ~ 9707
• 執行機構	臺北醫學大學醫學研究所		
• 年度	96 年	• 研究經費	1420 千元
• 研究領域	臨床醫學類		
• 研究人員	陳瑞明		
• 中文關鍵字	骨母細胞; 細胞存活; 細胞代謝; SATB2; 存活和代謝基因; RNAi; Dominant negative; EMSA; 報導基因分析		
• 英文關鍵字	Osteoblasts, Survival, Metabolism, SATB2, Survival and metabolism genes, RNAi, Dominantnegative, EMSA, Reporter gene assay		
• 中文摘要	骨頭結構(bone structure)的完整性，主要經由骨頭重整(bone remodeling)作用予以保持。骨母細胞 (osteoblasts)在骨頭生成過程中扮演重要角色，而不同區域(local)和系統(systemic)因子皆會調控骨母細胞活性。然而，細胞存活(survival)和代謝(metabolism)是決定骨母細胞功能完整性的兩個重要因子。於本實驗室先前對骨母細胞所做的序列研究中(NSC90-2314-B-002-196, NSC91-2314-B-038-031, NSC92-2314-B-038-010, NSC94-2314-B-038-013)已證實，氧化壓力(oxidative stress)會引起骨母細胞的凋亡(apoptosis)，而作用的分子機轉亦陸續在我們的研究中被闡明(Chen et al, 2002, Chen et al., 2005a, 2005b, and Ho et al., 2005)。在最近的研究中更進一步發現(NSC95-2314-B-038-029-MY3)，若前處理低濃度一氧化氮(nitric oxide；NO)可以保護骨母細胞免於氧化壓力所造成的傷害，而保護機轉則和誘導存活基因 Bcl-2 和 Bcl-XL 的表現，以及增加 Bcl-2 和 Bcl-XL 蛋白由細胞質轉位(translocate)到粒線體膜的量有關(Chang et al., 2006; Tai et al., 2006)。近來，本實驗室進行轉錄分析(transcriptional analysis)證實，轉錄因子 Runx2 和 GATA-3 可能牽涉到調控 Bcl-2 和 Bcl-XL 的基因表現。SATB2 是最近被發現的一種核基質結合區域蛋白(nuclear matrix-attachment protein)，其可以經由鍵結到 AT-rich 區域後，直接或經由協同其他轉錄因子而調控特定基因的表現。在我們的先期實驗中亦發現，SATB2 可能參與 NO 保護骨母細胞存活的機制中。先前研究也證實，SATB2 可以經由調控 osteocalcin 的表現而影響骨母細胞的分化。Osteocalcin 和骨母細胞的代謝有關。然而，有關 SATB2 在骨母細胞存活和代謝中可能扮演的角色，至今尚未被探討。研究骨母細胞存活和代謝的機制事關骨質的形成，將有助於瞭解骨科常見病症如骨質疏鬆症和風濕性關節炎可能的成因。本計劃是 3 年期		

	連續型研究計劃，將以初代培養(primary culture)大鼠頭蓋骨骨母細胞和人類骨癌細胞株(human osteosarcoma MG63 cell line)爲研究模式，探討核基質結合區域蛋白 SATB2 在骨母細胞存活與代謝中可能扮演的角色。此計劃的研究假說爲：SATB2 可經由與轉錄因子 Runx2 和 GATA-3 的協同作用，去調控存活基因 Bcl-2 family 和代謝基因 osteocalcin 的表現，並進而達到參與骨母細胞的存活與代謝作用。本計劃各年之研究假說和目標，分別敘述如下：計劃第一年：研究假說：SATB2 會參與骨母細胞存活與代謝作用。研究目標：1. 首先將測定骨母細胞在未處理、氧化壓力處理、NO 前處理或 dexamethasone 處理下， SATB2 於骨母細胞中可能產生的差異性表現(differential expression)，藉此以釐清 SATB2 與骨母細胞活性間的可能關聯性。 2. 再以 RNAi 技術將 SATB2 降低表現量(knockdown)的情況下，探討 SATB2 是否參 NO 保護骨母細胞免於氧化壓力傷害的過程中，以此釐清 SATB2 於骨母細胞存活作用中可能扮演的角色。3. 當 SATB2 以 RNAi 技術將其 loss of function 情況下，研究 SATB2 是否參與骨母細胞因 dexamethasone 刺激而礦物化(mineralization)的過程中，並以此釐清 SATB2 對骨母細胞代謝中可能扮演的角色。 4 於本年中亦將以 dominant negative 和 gene overexpression 技術，進一步探討 SATB2 分別在 loss of function 或 gain of function 情形下，於骨母細胞存活和代謝中可能扮演的角色計劃第二年：研究假說：SATB2 經由調控 Bcl-2 family 和 osteocalcin 基因表現以達參與骨母細胞存活與代謝的作用。研究目標：1. 以 RNAi 技術和 dominant negative plasmid construction 法降低細胞 SATB2 表現量，或將 SATB2 於骨母細胞中過量表現後，進一步測量 Bcl-2 和 Bcl-XL 於骨母細胞的表現量，藉此探討 SATB2 是否會調控 Bcl-2 和 Bcl-XL 基因的表現。 2. 當 SATB2 以 RNAi 技術和 dominant negative plasmid construction 法降低表現量，或將 SATB2 於骨母細胞中過量表現後，進一步測量 osteocalcin 於骨母細胞的表現量，藉此探討 SATB2 是否會調控 osteocalcin 基因的表現。 3. 以共軛焦顯微鏡法(confocal microscopy)和免疫點墨法(immunoblot)研究骨母細胞經藥物處理後，SATB2 於細胞的分佈。 4. 以 Electrophoretic mobility-shift assay (EMSA)分析法進一步探討，骨母細胞經藥物處理後，細胞核蛋白抽取物是否可以與 SATB2-DNA binding AT-rich region elements 進行鍵結。計劃第三年：研究假說：SATB2 會經由與 GATA-3 與 Runx2 的協同作用，進而專一性的會鍵結到 Bcl-2 family 和 osteocalcin 基因的 5』-end promoter regions，以調控這些基因的表現。研究目標：1. 以 PCR 法放大 Bcl-2、Bcl-XL 和 osteocalcin 基因的 5』-end promoter regions，並經進一步純化後，以 DNA sequencer 定序並確認所抽得的 DNA framents 確爲 Bcl-2、Bcl-XL 和 osteocalcin 基因的 5』-end promoter regions。 2. 以 exonuclease 將所抽得的 Bcl-2、Bcl-XL 和 osteocalcin 基因的 5』-end promoter regions 切爲不同長度的 DNA 片段，並經進一步純化後，將這些 DNA 片段轉殖(clone)到 plasmid 載體(vector)上。 3. 將已轉殖的 plasmid 載體 transform 到骨母細胞或 MG63 細胞，並以報導基因分析(reporter gene assay)法探討 GATA-DNA binding elements 於 Bcl-2、Bcl-XL 和 osteocalcin 基因的 5』-end promoter regions 的位置。 4. 以 site-directed mutation 法將 SATB2 上的 DNA-binding domain 進行氨基酸突變轉換，並以此探討 SATB2 對 Bcl-2、Bcl-XL 和 osteocalcin 基因的調控是否會受到影響。經由本計畫之執行，並結合本實驗室先前的研究成果，將對 SATB2 於骨母細胞中的表現，以及其於骨母細胞存活與代謝作用中的角色，有更深入的瞭解。更希望經由此一系統性的研究，能進一步闡明 SATB2 對於骨細胞的生理和病理作用，並有助於瞭解骨科相關病症的可能成因。
• 英文摘要	Bone structure is maintained by bone remodeling. Osteoblasts play a crucial role in mediating bone formation. A complicated

network of local or systemic mediators can modulate osteoblast activities. Cell survival and metabolism are two typical factors to determine osteoblast functions. Our previous studies (NSC90-2314-B-002-196, NSC91-2314-B-038-031, NSC92-2314-B-038-010, NSC94-2314-B-038-013) provided several lines of evidence to show that oxidative stress can induce osteoblast death via an apoptotic mechanism. At the same time, the possible signal-transducing mechanisms of osteoblast death had also been evaluated in our lab (Chen et al, 2002, Chen et al., 2005a, 2005b, and Ho et al., 2005). In the present study (NSC95-2314-B-038-029-MY3), our data further revealed that pretreatment with low nitric oxide (NO) can protect osteoblast from oxidative stress-induced apoptotic insults possibly through regulation of survival gene Bcl-2 and Bcl-XL expressions and their protein translocations from the cytoplasm to mitochondria (Chang et al., 2006; Tai et al., 2006). Recently, our transcriptional analyses showed that transcription factors Runx2 and GATA-3 may be involved in gene regulation of Bcl-2 and Bcl-XL. SATB2 is newly found protein which is attributed to a member of the nuclear matrix-attachment region proteins. SATB2 can bind to AT-rich regions to directly regulate certain gene expression. In addition, SATB2 has been reported to associate with other transcription factors to regulate gene expression. As done by Runx2 and GATA-3, the evidence based on gene analysis of SATB2 in osteoblasts revealed that this nuclear matrix-attachment region protein could participate in regulation of cell survival. A previous study has reported that both of SATB2 and Runx2 can regulate osteocalcin gene expression to promote osteoblast differentiation. Osteocalcin also contribute to osteoblast metabolism. However, the roles of SATB2 in osteoblast survival and metabolism and its possible molecular mechanisms are still known little. This is a 3-year-period project which is specified to evaluate the roles of nuclear matrix-attachment region protein SATB2 in osteoblast survival and metabolism using primary neonatal rat calvarial osteoblasts and human osteosarcoma MG63 cells as the experimental models. We hypothesized that SATB2 contributes to the regulation of osteoblast survival and metabolism via associating with transcription factors GATA-3 and Runx2 to promote transactive expressions of Bcl-2 family and osteocalcin. To verify our hypothesis, the sub-hypothesis and its specific aim for each year』s sub-project are designed and described as below: Sub-hypothesis-1: SATB2 is involved in osteoblast survival and metabolism. Aims: 1. To evaluate the relationship between SATB2 expression and osteoblast activities. The differential expressions of SATB2 in control, oxidative stress-, low NO-, or dexamethasone-treated osteoblasts are analyzed to achieve this aim. 2. To determine the roles of SATB2 in osteoblast survival. In this aim, SATB2 will be knocked down using RNAi to evaluate its effects on NO』s protection against oxidative stress-induced osteoblast apoptosis. 3. To validate the roles of SATB2 in osteoblast metabolism. SATB2 function will be lost using RNAi to determine the alternation of osteoblast mineralization under dexamethasone stimulation. 4. The roles of SATB2 will be further identified using the dominant negative and gene over expression methods. Sub-hypothesis-2: SATB2 can regulate osteoblast survival and metabolism through modulating Bcl-2 family and osteocalcin gene expressions. Aims: 1. To determine the effects of SATB2 on gene regulation of Bcl-2 and Bcl-XL in osteoblasts. When SATB2 is knocked down by RNAi or the dominant negative method or overexpressed, the expressions of Bcl-2 and Bcl-XL are determined. 2. To evaluate the effects of SATB2 on gene

	regulation of osteocalcin in osteoblasts. When SATB2 is knocked down by RNAi or the dominant negative method or overexpressed, the expressions of osteocalcin are determined. 3. To determine the localization of SATB2 in osteoblast nuclei, analyses of confocal microscopy and immunoblot are carried out. 4. Electrophoretic mobility-shift assay (EMSA) is carried out to further determine if the extraction of nuclear proteins in osteoblasts can specifically bind to the SATB2-DNA binding AT-rich elements. Sub-hypothesis-3: SATB2 associated with transcription factors Runx2 and GATA-3 can specifically bind to the DNA-binding elements in the 5』-end promoter regions of Bcl-2 family and osteocalcin genes in osteoblasts and then transactionally regulates these genes. Aims: 1. To clone the 5』-end promoter regions of Bcl-2,Bcl-XL, and osteocalcin genes using polymerase chain reaction. After purifying the fragments, the DNA sequences are confirmed using the DNA sequencer. 2. Using exonuclease to digest the 5』-end promoter regions into different lengths of fragments. After purification, the various DNA fragments are cloned into plasmid vectors. 3. Transforming these DNA-inserted plasmids into primary osteoblasts or human osteosarcoma MG63 cell line. And, the reporter gene assay is carried out to determine the AT-rich, Runx2-, and GATA-DNA binding sites in the 5』-end promoter regions of Bcl-2, Bcl-XL, and osteocalcin genes. 4. We will mutate the protein- or DNA-binding domains of SATB2 using the site-directed mutation method to evaluate the transactional effects of mutated SATB2 on gene regulation of Bcl-2, Bcl-XL, and osteocalcin genes.