行政院國家科學委員會專題研究計劃成果報告

E2F1 在細胞增殖、細胞凋亡及腫瘤形成中扮演的角色

Role of E2F1 in Cell Proliferation, apoptosis and tumorigenesis

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一、中文摘要

先前的動物模式研究顯示 Rb 蛋白被 T_{121} 致癌基因抑制會導致小鼠腦上皮細胞不正常增殖及 p53 依賴性細胞凋亡。若使 p53 失活則導致快速腫瘤生長，因為細胞凋亡會減少 85%。本研究分析 $T_{121}E2F1^{-/-}$小鼠，發現 E2F1 的缺失可導致 80%的細胞凋亡減少。此外，p53 下游基因的轉譯在這個模式中也受到破壞，顯示 E2F1 應作用在 p53 的上游。然而，E2F1 的缺失並不會使腫瘤加速生長，因為腫瘤細胞的增殖在 E2F1 缺失的情況下也被破壞了。由此可知，E2F1 是腫瘤細胞增殖及進行 p53 依賴性細胞凋亡所同時必需的，這也解釋了為什麼 E2F1 在實驗系統中可同時身為致癌基因與抑癌基因的明顯矛盾。

關鍵詞：E2F1、p53、細胞凋亡、增殖

Abstract

Previous animal model studies have shown that inactivation of the Rb proteins in mouse brain epithelium by the T_{121} oncogene induces aberrant proliferation and p53-dependent apoptosis. p53 inactivation causes aggressive tumor growth due to an 85% reduction in apoptosis. In this study, by analyzing $T_{121}E2F1^{-/-}$ mice, we found that E2F1 deficiency causes an 80% apoptosis reduction. In addition, transcriptional activation of p53 target genes is impaired in these mice, indicating that E2F1 acts upstream of p53. E2F1 deficiency does not accelerate tumor growth, however, because tumor cell proliferation is also impaired in the absence of E2F1. Thus, E2F1 is required both for tumor cell proliferation and p53-dependent apoptosis, explaining the apparent paradox that E2F1 can act as both an oncogene and a tumor suppressor in experimental systems.

Keywords: E2F1, p53, apoptosis, proliferation

二、緣由與目的

When activated by DNA damage, oncogene events, oxidation stress, and other aberrant cellular states, p53 can induce apoptosis, an activity that has been linked to its tumor suppressor capacity in animal models (1). In previous work, we developed a transgenic mouse model, TgT_{121}, in which epithelial brain tumors are initiated by inactivation of the Rb proteins with an N-terminal fragment of SV 40 T antigen (T_{121}). Under these conditions, the normally nondividing choroid plexus (CP) cells are induced to proliferate, resulting in the induction of p53-dependent apoptosis (2). Tumor growth in this model is slow because of high apoptosis level. When these mice are moved to p53 null background, apoptosis level is dramatically reduced and tumor growth is accelerated. This brain tumor model thus provides an excellent *in vivo* system in which to explore the downstream targets of Rb and the effectors of p53-dependent apoptosis.

Rb is regulated during the cell cycle by phosphorylation (3). At the molecular level, Rb

appears to act by modulating the activity of specific transcription factors, the best studied of which is E2F1. Phosphorylation of Rb results in the activation of E2F1, which is thought to drive the G1 to S phase transition. E2F1 activates the transcription of several S phase genes. However, it can also act as a transcription repressor when complexed with Rb (4). Despite the evidence that E2F1 is involved in cell cycle regulation, development proceeds normally in E2F1-deficient mice (5).

In addition to cell cycle regulation, E2F1 overexpression has been shown to trigger apoptosis in subsequent to S phase entry of quiescent cells (6,7). Of the five known mammalian E2Fs, the ability to induce apoptosis is unique to E2F1 (8). A role for E2F1 in apoptosis could explain the observation that mice deficient for E2F1 are prone to tumors with advanced age (5). However, a role for E2F1 in tumor suppression is difficult to reconcile with its apparent ability to drive the cell cycle and to act as an oncogene in cultured cells.

In this study, we examined the roles of E2F1 in p53-dependent apoptosis and tumor growth in the TgT_{121} transgenic mouse model described above. We examined tumor cell apoptosis and tumor growth in the presence and absence of E2F1 after interbreeding TgT_{121} mice with E2F1-deficient mice. We found that E2F1 has a clear role in the induction of p53-dependent apoptosis and, suprisingly, is also required for the tumor cell division cycle.

三、結果與討論

To directly test whether E2F1 is required for the induction of p53-dependent apoptosis, we generated TgT_{121} mice deficient in E2F1 by a series of backcrosses with $E2F1^{-/-}$ mice. Apoptosis within the CP of several TgT_{121} $E2F1^{+/+}$, TgT_{121} $E2F1^{+/-}$, and TgT_{121} $E2F1^{-/-}$ young mice (2-11 weeks) was measured by in situ TUNEL analysis. The apoptosis indices (AI) in control TgT_{121} $E2F1^{+/+}$ and TgT_{121} $E2F1^{+/-}$ littermates averaged 7.3 $\pm$ 1.25% and 7.02 $\pm$ 0.95%, respectively. However, the AI for TgT_{121} $E2F1^{-/-}$ mice was reduced to 1.79 $\pm$ 0.31%, about 80% reduction. This reduction is comparable, within experimental error, to the 85% apoptosis reduction previously observed upon p53 inactivation. Thus, the results show that E2F1 is required for p53-mediated apoptosis in this system.

To test whether E2F1 act in the p53 pathway and whether it act upstream p53, we examined the transcription of several p53 target genes such as p21, mdm2 and bax in CP by in situ hybridization. In the absence of E2F1, the level of p21 transcripts in CP was reduced by 89%. Similar reductions in mdm2 and bax were also caused by E2F1 deficiency. These results indicate that E2F1 lies upstream of p53.

To determine the overall impact of E2F1 deficiency on tumor growth, we compared the survival time and morphological development of CP tumors in TgT_{121} $E2F1^{+/+}$ and TgT_{121} $E2F1^{-/-}$ mice. TgT_{121} $E2F1^{+/+}$ mice developed slow-growing tumors, with a tumor survival t_{50} of 26 weeks. In TgT_{121} $E2F1^{-/-}$ mice, death was not substantially accelerated (t_{50} =20 weeks) and these mice died from hydrocephalus rather than tumor burden. Comparison of the size of CP tumor masses revealed that tumor masses in TgT_{121} $E2F1^{-/-}$ mice did not grow larger than those of TgT_{121} $E2F1^{+/+}$ mice. In contrast, CP of $TgT_{121}p53^{-/-}$ mice showed enlarged tumor masses by 4 weeks of age. Thus, although p53-dependent apoptosis was severely diminished in the absence of E2F1, this did not lead to accelerated tumor growth as occurs upon p53 inactivation.

Previous cell culture studies have shown that E2F1 can drive cells from G1 to S phase, yet animal studies clearly show that E2F1 is dispensable for normal cell cycle, as evidenced by normal development and survival of $E2F1^{-/-}$ mice. To test whether E2F1 is required for the induction and maintenance of aberrant CP cell

proliferation, the fraction of tumor cells in S phase was determined in the presence or absence of E2F1 by in situ immunodetection of BrdU incorporation. Compare TgT_{121} $E2F1^{+/+}$ and TgT_{121} $E2F1^{-/-}$ mice, the percentage of cells in S phase was clearly reduced in E2F1 deficient mice from 42% to 73% as in 6 weeks to 25 weeks of age. On average, the S phase fraction was reduced by 61% in the absence of E2F1. Since a decrease in the number of BrdU positive cells could result from either reduced cell cycle activity or a shortened S phase, the percentage of M phase tumor cells was also determined. M phase cells were detected by immunohistochemical staining of phosphory-lated histone H3. There was a significant reduction in the fraction of M phase cells in E2F1 deficient CP over time, which is parallel to the decrease in S phase. This demonstrates that E2F1 is required for efficient tumor cell proliferation and explains why overall tumor growth was not accelerated in $TgT_{121}E2F1^{-/-}$ mice, and thus why E2F1 is not a tumor suppressor in this system.

四、成果自評

Results from this study have been published (9). The research described above basically follows the outlines presented in the original project with a bit expansion from that. Generally speaking, this project has fulfilled the anticipated goals and is of academic value. It provides substantial evidence that E2F1 has a key role in p53-dependent apoptosis in vivo, and demonstrates that E2F1 is clearly required for efficient tumor cell growth. The study also indicates that E2F1 could be an important factor in the dynamics of the tumors and may be an appropriate target for specific drug therapy development.

五、參考文獻

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