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Identification of p53 regulation site on the p27 promoter

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Introduction

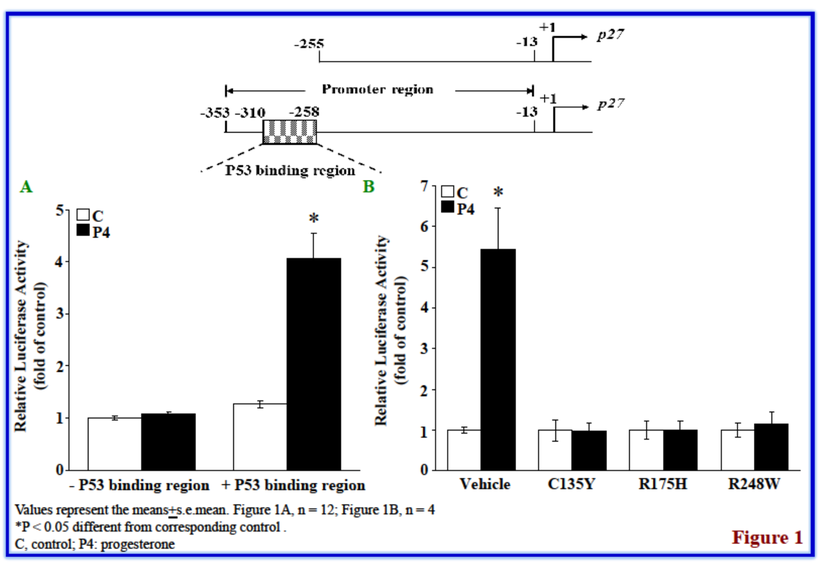
Angiogenesis is a crucial physiological phenomenon in female reproductive cycle. Our previous studies have shown that progesterone could inhibit proliferation of human umbilical vein endothelial cells (HUVEC) through a p53-dependent mechanism, by which the levels of p21 and p27 protein were increased, subsequently inhibiting the CDK2 kinase activity, and finally impaired the transition of the cell from the G1 phase to the S phase. While previous studies had clearly demonstrated that p53 protein directly activates p21 expression though binding onto the p21 promoter, the p53-regulated p27 gene expression has not been reported. Accordingly, the aim of this research was to investigate the precise binding domains of p53 protein on the p27 promoter. Luciferase assay showed that the potential p53 binding region spans on site 258 to site 310 upstream the start codon of the p27 gene. Within this range, there are three potential binding fragments with 70% similarity of p53 consensus binding domain and between each fragment is separated by less than 13 base pairs. Deletion or TCCT sequence replacement at the internal site of anyone of these fragments resulted in an irresponsiveness to progesterone treatment, suggesting that all these three fragments are essential for p53 protein to regulate the p27 promoter activity. Moreover, immunoprecipitation and chromatin-immunoprecipitation analysis demonstrated that both the formation of p53-progesterone receptor complex in the nucleus and the binding of progesterone receptor onto the p53 binding fragment of the p27 promoter were increased by progesterone treatment, suggesting that progesterone receptor might be also involved in the p53-regulated the p27 promoter activity. To our knowledge, this is the first demonstration that progesterone up-regulated p27 expression in HUVEC through a p53-dependent pathway..

Materials

Primary cells: HUVEC
Hormone: Progesterone (P4; 500 nM)
Dominant-negative p53 construct: C135Y; R175H; R248W
P27 promoter p53-binding site mutant: TCCT sequence replacement

Question I

Is p53 involved in the P4-induced increases of p27 promoter activity?



Values represent the means±s.e.mean. Figure 1A, n = 12; Figure 1B, n = 4
*P < 0.05 different from corresponding control .
C, control; P4: progesterone

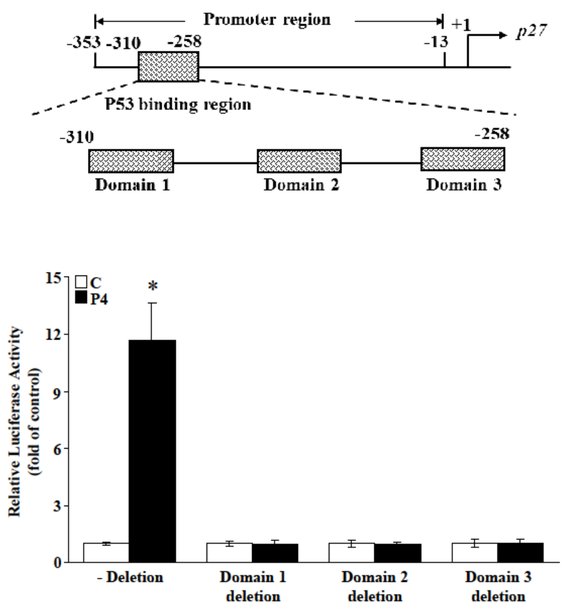
Figure 1

Result 1

Transfection with the p27 promoter constructs containing no potential p53 binding region (-258~ -310 fragment) into HUVEC caused a complete reduction of the P4-induced up-regulation of p27 promoter activity (Fig. 1A). Pre-transfection of HUVEC with the dominant-negative p53 constructs abolished the P4-induced increases of p27 promoter activity (Fig. 1B).

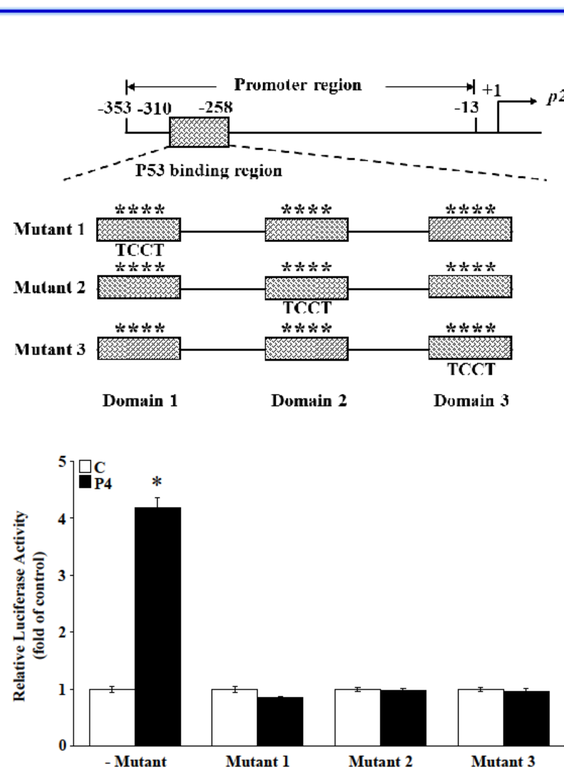
Question II

Are the potential p53 binding domains within the -258 ~ -310 fragment of p27 promoter required for the P4-induced increases of p27 promoter activity?



Values represent the means±s.e.mean. (n = 4).
*P < 0.05 different from corresponding control.
C, control; P4: progesterone

Figure 2



Values represent the means±s.e.mean. (n = 4).
*P < 0.05 different from corresponding control.
C, control; P4: progesterone

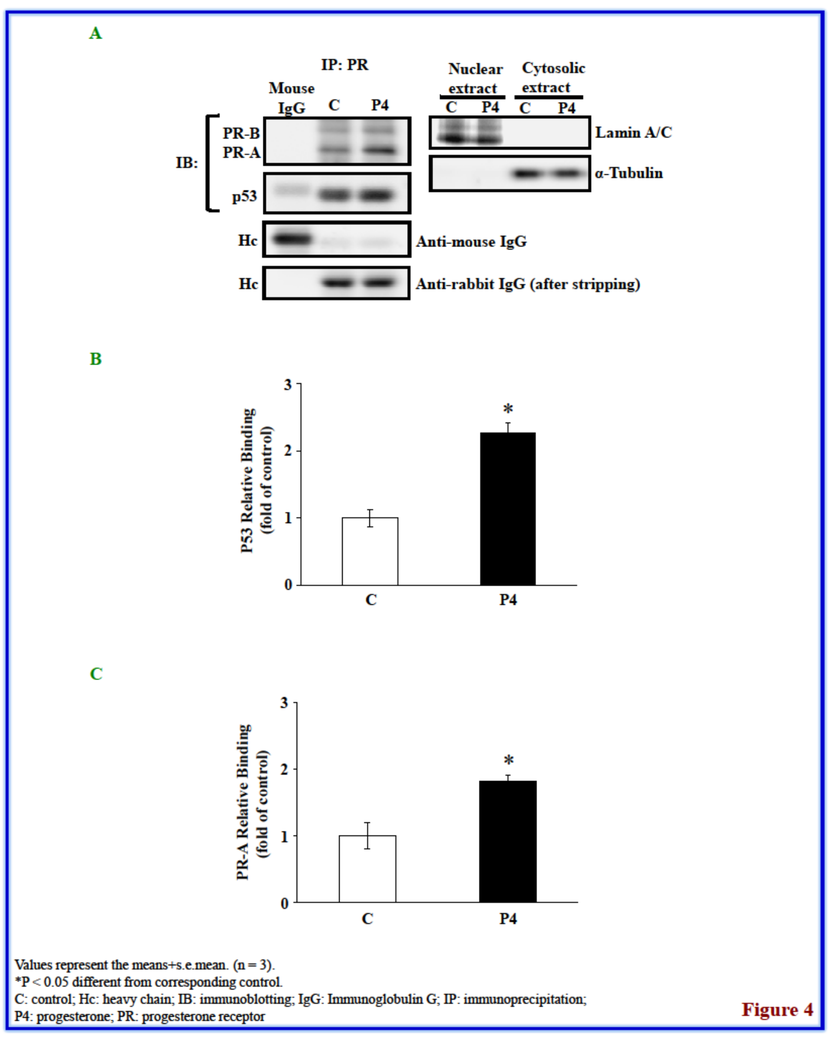
Figure 3

Result 2

Pre-transfection of HUVEC with the p27 promoter constructs containing deleted p53 binding domain (Fig. 2) or TCCT sequence replacement of p53 binding domain (Fig. 3) displayed a dramatic decrease in the P4-induced up-regulation of the p27 promoter activity.

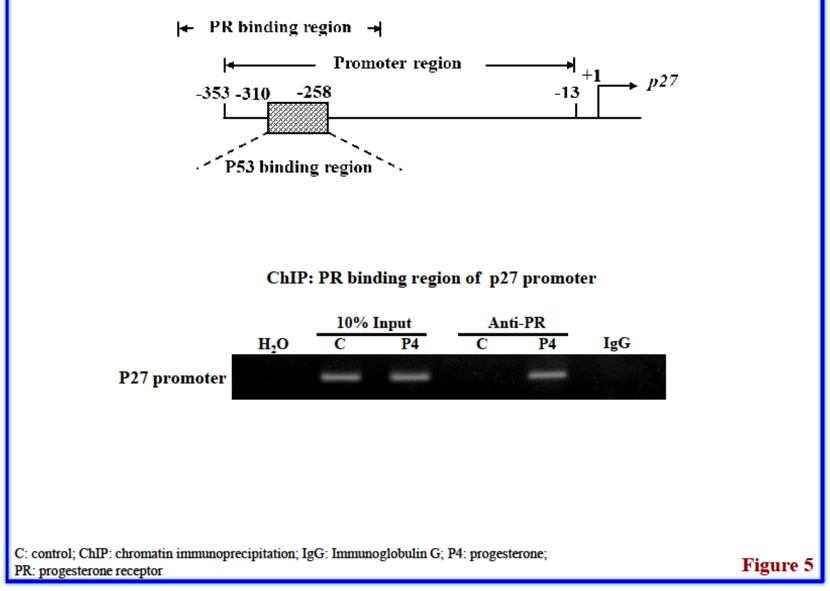
Question III

Is progesterone receptor involved in the P4-induced increases of p27 promoter activity?



Values represent the means+s.e.mean. (n = 3).
*P < 0.05 different from corresponding control.
C: control; Hc: heavy chain; IB: immunoblotting; IgG: Immunoglobulin G; IP: immunoprecipitation;
P4: progesterone; PR: progesterone receptor

Figure 4



C: control; ChIP: chromatin immunoprecipitation; IgG: Immunoglobulin G; P4: progesterone;
PR: progesterone receptor

Figure 5

Result 3

Immunoprecipitation analysis showed that treatment of HUVEC with P4 increased the formation of p53-PR complex in the nucleus (Fig. 4). ChIP assay indicated that P4 treatment increased the DNA binding activity of PR onto p27 promoter in HUVEC (Fig. 5).

Conclusion

These results reveal that P4-induced up-regulation of p27 expression is in a p53-dependent manner and suggest that PR might be involved in the regulation of p27 promoter activity.

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