# The Role and Mechanism of Nuclear Factor κB in HOC Angiogenesis

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**Abstract** **Background & Objective** It had been reported that nuclear factor κB(NF κB) play a role on angiogenesis in vitro, but its role in human ovarian carcinoma (HOC) angiogenesis had not been reported yet. The objective of this paper was to study the role and the mechanism of NFκB on HOC angiogenesis in Chick chorioallantcic membrane (CAM). **Methods** The HOC cell line TYK cells were implanted into 10-day CAM by $1\times10^6$/per egg to establish the HOC CAM model. 1) With 20 eggs, the levels of NFκB, integrin αVβ3, bFGF at 0h, 6h, 12h, 24h, 48h, 72h after cells implanted were determined with ELISA. 2) The relationship among NFκB, bFGF, αVβ3 and the effect of them on HOC angiogenesis in CAM were detected. Another 80 eggs were divided into 6 groups, Normal saline (NS) (control group, 16 eggs), anti-NFκB (group A, 16 eggs), anti-bFGF (group B, 16 eggs), anti-NFκB + anti-bFGF(group D, 8 eggs) were added respectively into CAM at 6h after cells implanted ,and then anti-integrin αVβ3 (group C, 16 eggs), anti-NFκB +anti-αVβ3(group E, 8 eggs) antibodies were added at 12h. 8 eggs from groups of NS、A、B and C were taken out to determine the levels of NFκB, αVβ3, bFGF at 48h after the antibodies were added. The other eggs were incubated up to 5 days, and the vessel area/area were detected with image analysis. **Results** 1) The levels of NF κB, bFGF increased significantly at 6h, and that of αVβ3 increase significantly at 12h after implantation($P<0.01$), the peaks of the three levels were reached at 48h ($P<0.01$), The levels of bFGF was higher significantly than those of NFκB and αVβ3 at each time ($P<0.01$), but there were no significant difference between NFκB and αVβ3 ($P>0.05$). 2) The levels of NFκB, bFGF and αVβ3 in groups A and B were lower significantly than that in NS group ($P<0.01$) And The level of αVβ3 in group C were reduced significantly compared with NS group ($P<0.01$), while the levels of NFκB and bFGF had no change between the two groups ($P>0.05$). The VA/A of the five groups of A, B, C, D and E were lower significantly than that of NS group ($P<0.05$) and that of D group were reduced significantly when compared with A group ($P<0.05$), but there were no significant difference among the four groups of A, B, C and E ($P>0.05$). **Conclusion** NF κB can stimulate the angiogenesis of ovarian carcinoma with bFGF by up regulate the expressions of αVβ3, bFGF. Which can be used as a marker of angiogenesis and a therapy target molecule of ovarian carcinoma.

**Key Words** NF κB; human ovarian carcinoma; Angiogenesis; Animal study

There were significant correlation between the new tumor angiogenesis and the growth, metastasis and prognosis of ovarian carcinoma, It had huge potential to inhibit tumor angiogenesis in the therapy of ovarian carcinoma[1,2] but the angiogenesis mechanism of ovarian carcinoma had not been reported yet. It had been report that nuclear factor kB had related to the angiogenesis in vitro[3-5], But that in vivo were reported very little[6], and had no reported in the ovarian carcinoma. The HOC CAM tumor and angiogenesis model were established with HOC cell line TYK cells in the study, in order to study the role of NFκB and their regulative role about bFGF、αVβ3 in the angiogenesis of ovarian carcinoms.

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## *MATERIAL AND METHODS*

### Experiment material

**Experiment animal and cell lines:** chicken

embryo: German YiLi chicken species, purchased from experiment hennery of Kunming. TYK: human ovarian cancer cell line, coming from the Tokyo medicine university of Japanese, provided by QiLu hospital of Shandong University.

**Experiment equipment** $CO_2$ culture box: German Heraeus company produced; Purify worktable SW-CJ-IF: The product of limited company product of air technique of An Tai in Suzhou, types YJ-1450; Galvanothermy constant temperature culture box: made in the scientific instrument pool factory in Hu Nan, 303 type; Image analyse system (Q970 type): experiment center of Yun Nan university, produced by Cambridge apparatus company of England; Cannon Camera: made in Japan; Low temperature high speed centrifuge: The German Heraeus company produced, Biofuge15R and Megafuge1.0R type respectively; convert discrepancy microscope: produced by Japan olympus company, BH-2 type; 96 formen enzyme mark board: made in American Nunc company; Enzyme mark automatism reading instrument: produced by American Biotcck company, BL340 type.

**Reagents** Mono-antibody to anti-NFκB, anti-bFGF were products of American Santa Craz Biotechnology Inc, purchased from gene limited company of Kunming; Mono-antibody to anti-integrinαVβ3: The product of PharMingen international, buy from Biology limited liability company in Shenzhen Jin mei; The RIPA solution of buffer, wrap, dilution, bottom and the terminate of the ELISA experiment were products of Sigma, buy from the Hua mei company. The normal saline produced by the first pharmacy factory of Yunnan.

**Experiment methods**

The establishment of human ovarian carcinoma angiogenesis model: TYK cell line cell grow in RPMI-1640 incubate fluid containing 15% calf serum, penicillin 100 u/ml, streptomycin 100 μg/ml, incubated in 37℃ constant temperature obturation, 5% $CO_2$ culture box. Index cell line TYK cells were implanted into 10-day CAM by $1\times10^9$/per egg by window.

The determination of the levels of NF κB、bFGF and αVβ3 of HOC angiogenesis in CAM: The CAM of 20 eggs were taken out at 0h, 6h, 12h, 24h, 48h, 72h after cells implanted, which were 2~3 piece of at each time. they were deep freezed liquid nitrogen after washed with sterile PBS, homogenized in RIPA buffer solution, 14000× g, 4℃ centrifuge 15 minutes, examining the total protein by Kamaslianglan method, the total concentration were 5.875 mg/ml、9.125 mg/ml、5.5 mg/ml、4.812 mg/ml、36.5 mg/ml、8.875 mg/ml、38.8 mg/ml respective at each point, which were diluted 4 mg/ml with PBS and determine the levels of NF κB、bFGF、αVβ3 by ELISA.

The effect of NF κB, bFGF and αVβ3 to HOC angiogenesis and tumor tissue in CAM: 80 eggs were divided into 6 groups, normal saline (NS) (control group, 16 eggs), anti-NFκB (A group, 16 eggs), anti-bFGF (B group, 16 eggs), anti-NFκB + anti-bFGF(D group, 8 eggs) were added respectively into CAM at 6h time point after cells implanted, and then anti-integrin αVβ3 (C group, 16 eggs), anti-NFκB +anti- αVβ3 (E group, 8 eggs) antibodies were added at 12h. 8 eggs from NS, A, B, C groups were taken out to determine the levels of NFκB, αVβ3, bFGF at 48h after the antibodies were added. The other eggs were incubated up to 5 days, and the vessel number, vessel area and vessel area/area were detected with image analysis, observing the change of angiogenesis and tumor tissue.

**The data processing**

The data were described in means±standard ($\overline{X}\pm$ S)and analyzed with least significant difference method of single factor square difference in the study with SPSS9.0 Stat software package. The test level was $\alpha=0.05$.

## *RESULTS*

**The Levels of NF κB、 bFGF and αVβ3 of HOC Angiogenesis in CAM**

The levels of NFκB, bFGF increased significantly at 6h, and that of αVβ3 increase significantly at 12h after implantation ($P<0.05$), the peaks of the three levels were reached at 48h ($P<0.01$), The levels of NF κB、αVβ3 at fastigium were 4 times and that of bFGF were 2 times the level at beginning. There were no significant change of the levels of αVβ3、bFGF、NFκB during 48~72h. The levels of bFGF was higher significantly than those of NFκB and αVβ3 at each time ($P<0.01$), but there were no significant difference between NFκB and αVβ3 ($P>0.05$) (see table 1).

**The Relationship among NF κB, bFGF and αVβ3 Of HOC Angiogenesis in CAM**

Control group, group A and group B were added into NS, anti-NFκB, anti-bFGF antibodies respectively into CAM at 6h after cells implanted, and then anti-integrin αVβ3 antibodies were added at 12h after the TYK cell lines implanted into CAM.

The levels of NFκB, bFGF and αVβ3 in group A and B were lower significantly than NS group ($P$<0.01). And The level of αVβ3 in group C were reduced significantly compared with NS group ($P$<0.01), while the levels of NFκB and bFGF had no change between the two groups at 48h. ($P$> 0.05). (see table 2)

**The Effect of NFκB、 bFGF、 αVβ3 on HOC Angiogenesis and Tumor Tissue in CAM**

Corresponding antibodies were added in each groups after the TYK cells were implanted into CAM, incubating 5 days. The growth of tumor angiogenesis and the tumor areas were determined by computer image analyse system. The results was show in table 3.

The number, tissue, vessel area/CAM area and T of the five groups of A,B, C, D and E were lower significantly than that of NS group ($P$<0.05), and that of D group were reduced significantly when compared with A group ($P$<0.05), but there were no significant difference among the four

**Table 1** the Levels of NF κB、bFGF and αVβ3 of HOC Angiogenesis in CAM

| Time point (h) | NF κB | bFGF | αVβ3 |
|---|---|---|---|
| 0 | 0.053±0.004 | 0.631±0.040[b] | 0.069±0.019 |
| 6 | 0.185±0.006[a] | 0.771±0.160[ab] | 0.120±0.010[a] |
| 12 | 0.216±0.020[a] | 1.064±0.050[ab] | 0.231±0.020[a] |
| 24 | 0.261±0.010[a] | 1.642±0.063[ab] | 0.276±0.020[a] |
| 48 | 0.337±0.010[a] | 1.639±0.014[ab] | 0.349±0.010[a] |
| 72 | 0.313±0.007[a] | 1.648±0.030[ab] | 0.347±0.010[a] |

[a,b]$P$<0.01, a: VS 0h, b: VS αVβ3

**Table 2** the Relationship among NF κB, bFGF and αVβ3 of HOC Angiogenesis in CAM

| Group | Expression | | |
|---|---|---|---|
| | NF κB | bFGF | αVβ3 |
| NS | 0.329±0.01 | 1.591±0.13 | 0.322±0.01 |
| A | 0.098±0.01* | 1.106±0.33* | 0.138±0.03* |
| B | 0.156±0.02* | 0.412±0.01* | 0.114±0.01* |
| C | 0.324±0.01 | 1.497±0.06 | 0.119±0.02* |

VS NS, *$P$<0.01。

**Table 3** the Effect of NFκB、bFGF、αVβ3 to HOC Angiogenesis and Tumor Tissue in CAM

| Group | N | N | A(mm²) | VA/A | T(mm²) |
|---|---|---|---|---|---|
| NS | 8 | 42.20±15.32 | 26.15±8.35 | 66.62±17.64 | 34.26±15.83 |
| A | 8 | 19.60±13.98[a] | 10.26±5.38[a] | 26.10±13.71[a] | 12.90±11.20[a] |
| B | 8 | 20.20±4.92[a] | 13.39±1.52[a] | 34.12±4.85[a] | 10.72±2.82[a] |
| C | 8 | 16.80±0.71[a] | 10.02±4.48[a] | 25.50±11.41[a] | 8.25±4.15[a] |
| D | 8 | 8.12±3.24[ab] | 5.16±1.27[ab] | 14.32±3.11[ab] | 4.23±1.37[ab] |
| E | 8 | 17.61±6.23[a] | 9.59±2.94[a] | 24.36±4.95[a] | 10.65±4.26[a] |

[a,b]$P$<0.05, a:Vs NS, b:VS A; N: number; A: vessel area; VA/A: vessel area/CAM area, T: tissue

groups of A, B, C and E ($P$>0.05).

## DISCUSSION

NFκB is a sort of poly-function transcription factor [7], which involved in tumor angiogenesis in multi-process[8,9]. Yoshida[3] reported that there were potential regulative role of NFκB to the expression of bFGF. Sharma[10] reported that integrin αV was the target molecule of NFκB, NFκB restrained the growth of endothelium cell by αVβ3, which indicated NFκB played the regulative role of endothelial cell wither in angiogenesis.

There were expressions of NFκB、bFGF 与 αVβ3 to a certain in 10 days CAM, t he levels of NF κB, bFGF increased significantly at 6h, and that of αVβ3 increased significantly at 12h after implantation, the peaks of the three reached at 48h, there were no significant change during 48~72h. The levels of bFGF was higher significantly than those of NFκB and αVβ3 at each time, but there were no significant difference between NFκB and αVβ3.

The expressions of NF κB、bFGF and αVβ3 were restrained by themselves antibodies showing the three antibodies were availability for sure., the antibody of NF κB can restrain the expressions of bFGF and αVβ3, the antibody of bFGF can restrain the expressions of NFκB、αVβ3 significantly, But the antibody of αVβ3 had no effect on the expression of NFκB, and bFGF. The three antibodies all can inhibited the growth of HOC CAM angiogenesis and tumor tissue, but there were no significant difference in the suppression ability among the three antibodies. When the antibodies of NFκB and bFGF were used combined, the suppression ability to the growth of HOC CAM angiogenesis and tumor tissue boost up significantly, which showed that they have cooperation with each other.

Combined with the reports before, we speculated it was an important way to start up the angiogenesis of ovarian carcinoma by bFGF stimulating the expression of NFκB、αVβ3, and NFκB promote the angiogenesis growth by upregulating the produce of αVβ3、bFGF. We supposed that NF κB、bFGF and αVβ3 begin to express at the time of startup and they interact, inter-activate during the course of angiogenesis. The bFGF come into being early and high concentration, which stimulated the expression of NFκB、αVβ3, and NF κB positive regulated the engender of bFGF、αVβ3. In this way, increased bFGF and NF κB promoted the forming of angiogenesis together, bFGF expedite the proliferation of blood vessel endothelium cells, and NFκB inhibit the cells wither byαVβ3, so they have the cooperation role. αVβ3 may be an essential step in the angiogenesis of NFκB, so they have no cooperation effect.

In a word, NFκB、bFGF and αVβ3 formed the immensity cancer vessel according cascade response, NFκB facilitated the form of ovarian carcinoma angiogenesis by up-regulating the expression of bFan adn αVβ3, which not only be taken as the marker of angiogenesis, but also become the therapy target.

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