POSTERS

P4 - EFFETS DU TGFβ1 (TRANSFORMING GROWTH FACTOR) SUR LA DIFFÉRENCIATION DES CELLULES SOUCHES ENDOMETRIALES HUMAINES

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RÉSUMÉ
Introduction: Nous assistons aujourd'hui à l'émergence de l'ingénierie tissulaire qui devraient changer le visage de la pratique odontologique. Des recherches ont montré qu'il était possible de reconstruire une dent à partir de cellules souches de pulp dentaire. Mais les cellules souches dentaires ont des limites. Afin de palier à ce problème, on s'intéresse désormais à l'identification des autres cellules adultes cultivables et capables de former dentine. Dans cette étude nous voulions investiguer les effets du TGFβ1 (Transforming Growth Factor) sur la différenciation odontoblastique des cellules souches endométriales.

MATÉRIELS ET MÉTHODES
Les cellules souches endométriales humaines ont été isolées par digestion enzymatique. Pour induire la différenciation des cellules souches en odontoblasts, le TGFβ1, les protéines non collagéniques de la dentine (DNCP) et TGFβ1+ DNCP ont été utilisé. Les résultats ont été évalués par l'activité de la phosphatase alcaline (ALP), des dépôts de calcium au rouge S d'alizarine, immunocytochimie pour la détection des marqueurs odontogeniques (la protéine de la matrice dentinaire-1 (DMP-1) et la sialoprotéine dentinaire (DSP)) et Western blotting.

Résultats
Coloration au rouge S d'alizarine, met en évidence la différenciation odontoblastique des cellules souches endométriales des groupes DNCP et TGFβ1+ DNCP. Les résultats montrent également une augmentation significative de l'activité de la phosphatase alcaline en présence du DNCP et TGFβ1+DNCP ($P<0.01$). D'ailleurs, les résultats d'immunocytochimie et western blotting ne montrent pas expression des marqueurs odontogeniques dans le group TGFβ1 .

CONCLUSIONS
Les résultats montrent que le TGFβ1 seul, n'a pas de la capacité d'induire et de différencier les cellules souches endométriales. Nous pouvons suggérer que les protéines non collagéniques de la dentine sont nécessaires pour présenter le signal au cellules.

MOTS-CLÉS
Différenciation ; Cellule souche ; Endomètre ; Odontoblasts

POSTERS

P4 - EFFECTS OF TRANSFORMING GROWTH FACTOR β1 (TGFβ-1) ON THE IN VITRO DIFFERENTIATION OF HUMAN ENDOMETRIAL STEM CELLS.

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KEY WORDS
TGFβ1, Endometrial stem cells, Differentiation, Dentin non-collagen proteins.

INTRODUCTION
Human endometrial stem cells has been isolated and identified in uterine endometer. These cells have the capacity to differentiate into derivatives of all 3 germ-layers, they are similar to dental pulp because of co-expression of CD146 and PDGF-receptor β (PDGF-Rβ) markers, they can generate new vessels and they can be obtained by a simple, safe and painless procedure (1-3). Evidence accumulated from above studies has opened up the possibility of using endometrial stem cells in the stem-cell-based tissue engineering of dentin (4).
Growth factors are implicated in mediating many of the key procedures in tooth morphogenesis and differentiation (5). Among all the growth factors, TGFβ1 has received particular consideration (6). Other studies confirmed that dentin non collagenous proteins (DNCPs) are implicated in the dentin regeneration (7). However, the synergistic effects of DNCP and TGFβ1 on the differentiation of endometrial stem cells are not yet elucidated. An effort was made in this study to assess the interaction pattern of these two materials.

MATERIALS AND METHODS
Endometrial tissues were collected from ovulating women at the Gynecology and Obstetrics Department of Vali-e-Asr Hospital, and used with the patients' informed consent. The endometrial stem cells were then separated from the biopsies and subjected to flow cytometry analysis, osteogenic and adipogenic differentiation.
DNCPs were extracted as previously reported by Smith (8). Passage 4 cells were seeded into 24- well plates. The medium contained Dulbecco modified Eagle medium (DMEM), 10% Fetal Bovine Serum (FBS), and 1% 100X antibiotic (all from (GIBCO BRL, Grand Island, N.Y., USA)). The experiments were divided into four groups: (1) group A: medium was supplemented with TGFβ-1 (2 ng/ml) and DNCP (250 ng/ml); (2) group B: only DNCP (250 ng/ml) was added to the medium; (3) group C: only TGFβ-1 (2 ng/ml) was added to the medium (4) control group in which TGFβ-1 and DNCP were absent from the medium. For Alkaline phosphatase (ALP) activity assay, cells were seeded into 96-well plates and incubated for 12 h, and divided into four groups as described above (n = 6). The ALP activity of the cells at day 3, 5, 7 and 14 was detected using an ALP assay kit according to the manufacturer's instructions. The results were measured spectrophotometrically at 405 nm and indicated by OD values. After the endometrial stem cells were induced by DNCP and TGFβ1 for 21 days, alizarin red staining, Immunostaining and western blotting with antibodies against DSP and DMP-1 was performed. Finally, statistical analyses of the data were performed by two way ANOVA and Tukey test ($P < 0.01$).

RESULTS

he endometrial adult stem cells strongly expressed CD105 and CD90, the putative marker of mesenchymal stem cells. While CD31 and CD34 did not express in these cells. Also, osteogenic and adipogenic differentiation of endometrial stem cells, were confirmed with alizarin red and oil red staining.

Augmentation of ALP activity in the DNCP and DNCP + TGFβ1 group was significant at day 5, 7 and 14 ($P < 0.01$). Moreover, a higher activity was presented in DNCP and DNCP + TGFβ1 groups, as compared with that in TGFβ1 and control groups ($P < 0.01$) (Fig.1).

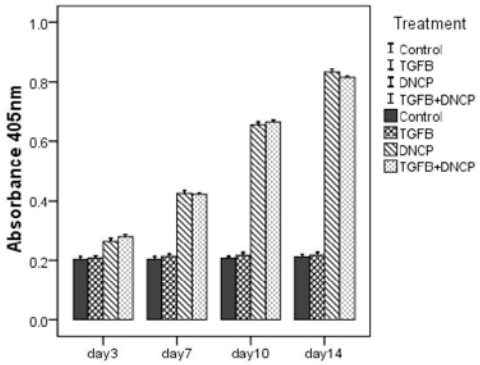

Fig. 1. ALPase activity changes in the control and induction groups (OD value at 410 nm, ± SD, n = 6)

Mineralized nodules were detected in the continuously cultured cells induced by DNCP and DNCP + TGFβ1 at day 21. The TGFβ1 and control groups did not form calcium nodules (Fig. 2c- d).

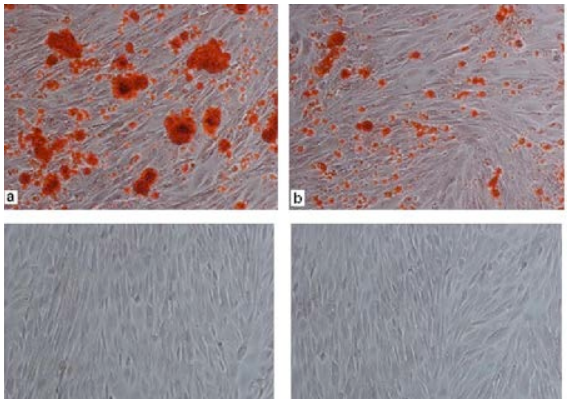

Fig. 2. mineralized nodules in the DNCP group and DNCP + TGFβ1 group (a&b), no nodule in the TGFβ1 or the control group (c&d).

The cells induced by DNCP and DNCP + TGFβ1 demonstrated positive expression of odontoblastic markers with immunocytochemistry (Fig. 3a-b). The TGFβ1 and control group demonstrated negative expression of these markers. These findings were confirmed with western blotting

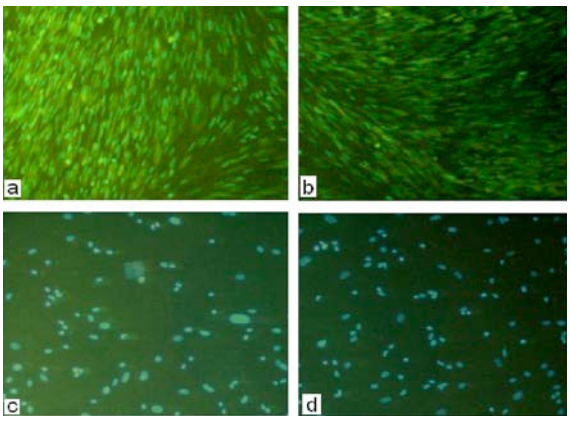

Fig. 3. positive expression of odontoblastic markers in the DNCP group and DNCP + TGFβ1 group (a&b), negative expression in the TGFβ1 or the control group (c&d).

DISCUSSION

To direct the differentiation of endometrial stem cells in vitro, we examined the effects of the TGFβ1 on these cells. Our results demonstrated that increase of ALP activity, after the administration of DNCP, TGFβ1 and DNCP + TGFβ1, was time-dependent which was consistent with previous studies (9). However, increase of the ALP activities in the TGFβ1 group was not significant, implying the necessity of presence of protein matrix for differentiation of endometrial stem cells. In the present work in contrast to other studies, the increase of the ALP activities in the TGFβ1 group was not significant, the mineralized nodules were not formed in the TGFβ1 group and neither DSP/DMP-1 proteins were expressed in the TGFβ1-treated cells. These results were not consistent with previous studies about the stimulatory effects of the TGFβ1 on the differentiation of stem cells (10, 11). This may due to several differences between these studies, including the source of stem cell. Our results demonstrate that the combination of DNCP and TGFβ1 is necessary for both the morphological and functional differentiation of endometrial stem cells in vitro.

CONCLUSIONS

In summary, it seemed that TGFβ1 did not play important roles in the in vitro differen-

tiation of endometrial stem cells into odontoblast cells. Animal studies may be required for investigation of the effect of these two materials on the differentiations of endometrial stem cells.

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