壁報論文作品欣賞 醫院組 第一名

Isolation of Mesenchymal Stem Cells from Human Deciduous Teeth Pulp

Aileen I Tsai1, Tzung-Hai Yen2

1Department of Pediatric Dentistry, Chang Gung Memorial Hospital , Linkou, Taiwan;

2Kidney Research Center, Chang Gung Memorial Hospital, Linkou, Taiwan

Abstract

長庚紀念醫院 - 蔡蔭玲 醫師

This study aimed to identify predictors of success rate of mesenchymal stem cell (MSC) isolation from human deciduous teeth pulp. A total of 161 deciduous teeth were extracted at the dental clinic of Chang Gung Memorial Hospital. The MSCs were isolated from dental pulps using a standard protocol. In total, 128 colonies of MSCs were obtained and the success rate was 79.5%. Compared to teeth not yielding MSCs successfully, those successfully yielding MSCs were found to have less severe dental caries (no/mild-to-moderate/severe: 63.3/24.2/12.5% versus 12.5/42.4/42.4%, $P<0.001$) and less frequent pulpitis (no/yes: 95.3/4.7% versus 51.5/48.5%, $P<0.001$). In a multivariate regression model, it was confirmed that the absence of dental caries (OR=4.741, 95% CI=1.564–14.371, P=0.006) and pulpitis (OR=9.111, 95% CI=2.921–28.420, $P<0.001$) were significant determinants of the successful procurement of MSCs. MSCs derived from pulps with pulpitis expressed longer colony doubling time than pulps without pulpitis. Furthermore, there were higher expressions of pro-inflammatory cytokines [interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1, $P<0.01$), and innate immune response [toll-like receptor (TLR) 1 and 8, $P<0.05$; TLR2, 3 and 6, $P<0.01$] in the inflamed than non-inflamed pulps. Therefore, a carious deciduous tooth or tooth with pulpitis was relatively unsuitable for MSC processing and isolation.

Introduction

The presence of stem cells in the dental pulp of a deciduous tooth is an exciting new finding that has significant meaning for the pediatric dental practitioners. When compared to other types of adult stem cells, obtaining stem cells from teeth is ethically non-controversial, non-invasive, less dependent on timing, and far less expensive.

Every child loses deciduous teeth, which creates the perfect opportunity to recover and store this source of stem cells. Stem cells from teeth replicate at a faster rate and for a longer period of time than do stem cells harvested from other tissues of the body . Another advantage of dental stem cells is that there is more than one opportunity to harvest teeth stem cells from deciduous teeth. The problem is, since pulpitis can be caused by extensive dental caries, it is unknown whether the presence of caries or pulpitis could affect the harvesting of MSCs from deciduous teeth. Our previous survey also indicated a high level of untreated dental caries (56%) among children less than 6 years of age.

Several studies have investigated whether dental pulp stem cells (DPSCs) isolated from teeth with pulpitis can survive and retain their tissue regenerative potential. Alongi et al showed that inflamed dental pulps expressed higher levels of stromal cell-derived factor-1 (STRO-1), CD90, CD105, and CD146 compared to normal pulps. Pereira et al demonstrated that the morphology, proliferation rate and differentiation potential of DPSC from inflamed pulps were similar to the observed in normal pulps.

The objective of this study was to identify predictors of success rate of MSCs isolation from these deciduous teeth pulp. It was an intriguing question with clinical implications, and that was how the condition of the tooth and/or pulp affected the ability to isolate MSC populations.

Methods and Materials

A total of 161 deciduous teeth were extracted at the pediatric dental clinic of Chang Gung Memorial Hospital. Data were obtained for each patient regarding the following parameters: age, sex, type of teeth, underlying diseases, interval between extraction to culture, severity of dental caries, and presence or absence of pulpitis.

Deciduous teeth were extracted and transported in Dulbecco's phosphate buffered saline solution containing 300 units/mL penicillin and 300 μg/mL streptomycin . The dental pulp was then minced and cultured at 5% CO2 atmosphere under 37C in a 35-mm culture disk containing passage 0 medium with α-modified Eagle’ s medium.

The passage 0 generation cells were grown in media until 90% confluence was achieved and reseeded at 10 × 103, 5 × 103, and 1 × 103 cells into new culture disks and incubated for 7–14 days. The doubling time was calculated using on-line formula (web address: www.doubling-time.com/compute.php).

For adipocytic differentiation, the passage 3 cells were plated in a culture disk at a concentration of 1×104 cells. They were then observed under a microscope for fat deposition.

In this study, the extraction of teeth and recording of tooth status, tooth type, tooth number, and general information of each patient were performed by a pediatric dentist. The severity of dental caries was classified according to the criteria of the International Caries Assessment and Detection System . In this study, teeth were categorized into 3 levels according to the severity of caries: (1) sound, teeth with no evidence of treated or untreated clinical caries; (2) mild to moderate caries, teeth showing obvious cavitation or those that had been restored with dental materials; and (3) severe caries, teeth that were treated with pulpotomy or pulpectomy. Four types of deciduous teeth were collected: incisors, canines, molars, and supernumerary teeth. The pulpal status was classified as (1) no pulpitis: sound or restored teeth, and (2) pulpitis: teeth with pulpectomy or pulpotomy. The pulpal treatments were confirmed by clinical examination and dental radiography.

Chi-square or Fisher exact test was used for categorical variables. To control the confounding factors, a multivariate logistic regression analysis (stepwise backward approach) was performed to analyze the significant covariates ($P < 0.10$) identified on simple logistic regression. The criterion for significance was a 95% confidence interval (CI) . Statistical analyses were performed using IBM SPSS Statistics Version 20.

Results

Most of the MSCs were spindle-shaped and fibroblast-like, while some were cuboidal or polygonal in appearance. The differentiation studies demonstrated that the MSCs could be sub-passaged and differentiated in vitro into a variety of cells of the mesenchyme lineages, such as osteoblasts, chondrocytes and adipocytes . Immunophenotype was ascertained by flow cytometry analysis. The analysis showed that MSCs were positive for the expressions of CD73, CD90, STRO-1, and CD44, but negative for the expressions of CD45, CD34, CD19, and HLA-DR. In all, 128 colonies of MSCs were obtained from 161 deciduous teeth. The success rate was 79.5% (Table 1). Those successfully yielding MSCs were found to have less severe dental caries and less frequent pulpitis. Multivariate logistic

regression analysis showed that the absence of dental caries (OR = 4.741, 95% CI = 1.564 – 14.371, P = 0.006) and pulpitis (OR = 9.111, 95% CI = 2.921 – 28.420, P < 0.001) were significant determinants for the successful isolation of MSCs (Table 2). The data suggest that a caries-free tooth or a tooth free of pulpitis had 4.741-fold or 9.111-fold greater chance of yielding a successful harvest of MSCs than a tooth with caries or pulpitis, respectively.

	Total (N = 161)	Successful isolation of MSCs	Unsuccessful isolation of MSCs	P
		(N = 128)	(N = 33)	
Age, year	8.590 2.782	8.631 2.885	8.431 2.373	0.715
Male, n (%)	115 (71.4)	945 (74.2)	20 (60.6)	0.123
Interval between extraction to culture, day	1.652 3.469	1.914 3.845	0.636 0.381	0.059
Type of teeth				0.219
Incisor, n (%)	31 (19.3)	26 (20.3)	5 (15.2)	
Canine, n (%)	21 (13.0)	16 (12.5)	5 (15.2)	
Molar, n (%)	61 (37.9)	44 (34.4)	17 (51.5)	
supernumerary, n (%)	48 (29.8)	42 (32.8)	6 (18.2)	
Severity of dental caries				<0.001***
No, n (%)	86 (53.4)	81 (63.3)	5 (12.5)	
Mild-to-moderate, n (%)	45 (28.0)	31 (24.2)	14 (42.4)	
Severe, n (%)	30 (18.6)	16 (12.5)	14 (42.4)	
Pulpitis				<0.001***
No, n (%)	139 (86.3)	122 (95.3)	17 (51.5)	
Yes, n (%)	22 (13.7)	6 (4.7)	16 (48.5)	

▲TABLE 1
Baseline characteristics of the collected deciduous dental pulps (N = 161)

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Absence of dental caries	9.651	3.490–2689	<0.001	4.741	1.564–14.371	0.006
Absence of pulpitis	19.137	6.586–55.607	<0.001	9.111	2.921–28.420	<0.001

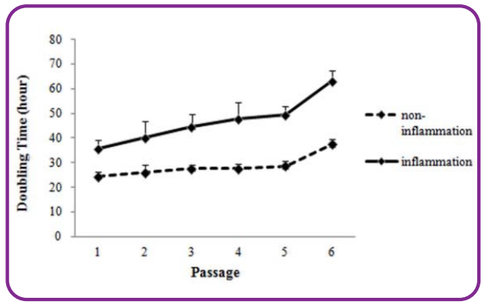
◀TABLE 2
Logistic regression analysis for successful isolation of MSCs derived from deciduous dental pulp (N = 161)

Discussion

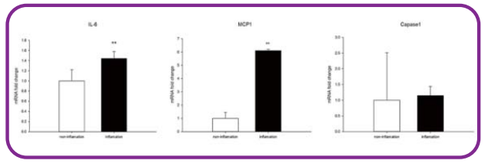
This clinical study, based on a considerable number of human samples (N = 161), provided evidence that a carious deciduous tooth or tooth with pulpitis was relatively unsuitable for MSC processing and isolation.

We found that deciduous teeth successfully yielding MSCs had less severe dental caries (P < 0.001) and less frequency of pulpitis (P < 0.001) than those not yielding MSCs. Our analysis revealed that MSCs derived from pulps with pulpitis expressed longer colony doubling time than pulps without pulpitis (FIGURE 1). Furthermore, deciduous pulps with pulpitis suffered higher expressions of pro-inflammatory cytokines (IL-6 and MCP-1), and innate immune response (TLR1, 2, 3, 6 and 8) than pulps without pulpitis (FIGURE 2 and 3).

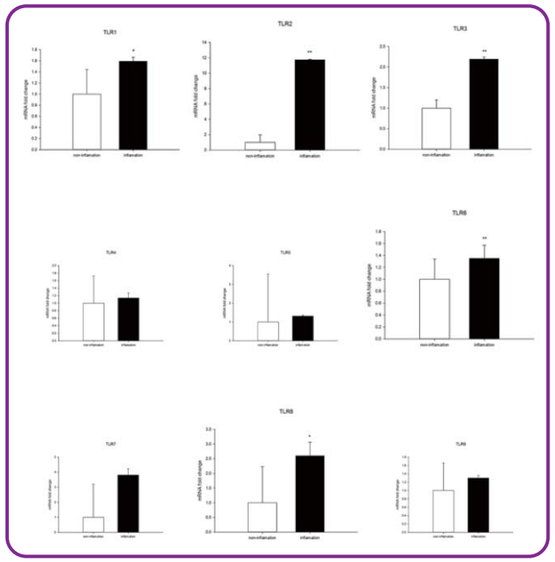
Contrary to our expectations, no significant differences were noted between the successful and unsuccessful MSC isolation groups with respect to the type of teeth used (Table 2). However, the ability of SHED and supernumerary-teeth DPSCs to differentiate into osteogenic, adipogenic, and chondrogenic lineages was similar. In particular, the population doubling time of supernumerary DPSCs increased while that of SHED remained nearly unchanged. Therefore, the team concluded that both supernumerary teeth and deciduous teeth share many characteristics, such as the ability to yield highly proliferative clonogenic cells



▲FIGURE 1
Colony doubling time



▲FIGURE 2
Pro-inflammatory cytokines



▲FIGURE 3
Innate immune response

with an immunophenotype similar to that of MSCs, although they are inferior to exfoliated deciduous teeth in terms of long-term banking.

Similarly, the successful and unsuccessful MSC isolation groups did not differ with respect to patient age (Table 1). The study revealed that DPSCs isolated from the juvenile donors displayed increased proliferation and decreased osteogenic differentiation ability than those isolated from adult DPSCs.

Conclusions

In summary, the data suggest that a carious deciduous tooth or tooth with pulpitis was relatively unsuitable for MSC processing and isolation. This observation is particularly important for dental stem cell banking and regenerative dentistry. Nevertheless, whether or not cell can be isolated is one question, the cell population end up with following the isolation is the more significant question.

長庚紀念醫院 Chang Gung Memorial Hospital

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TABLE 2 Logistic regression analysis for successful isolation of MSCs derived from deciduous dental pulp (N = 161)

Variable	Univariate analysis			Multivariate analysis		
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Absence of pulpitis	19.137	6.586–55.607	<0.001	9.111	2.921–28.420	<0.001

FIGURE 1 Colony doubling time

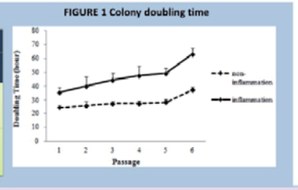


FIGURE 2 Pro-inflammatory cytokines

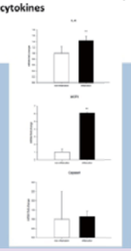
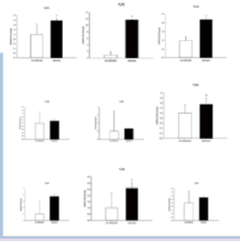


FIGURE 3 Innate immune response



壁報論文作品欣賞 醫院組

第二名

EMD與Vitamin C對牙周韌帶衍生細胞礦化相關mRNA表現的調節

Regulation of mineralization-related mRNA expressions by enamel matrix derivative and vitamin C in human periodontal ligament derived cells

梅至君　洪祥熙　長庚紀念醫院林口醫學中心牙科系牙周病科
Mei Chih-Chun; Hong, Hsiang-Hsi Department of Periodontics, Section of Dentistry, Chang Gung Memorial Hospital Linkou Medical Center, and Chang Gung University. Taoyuan, Taiwan

長庚紀念醫院 - 梅至君 醫師

Background

The mRNAs of core-binding factor subunit alpha-1 (Cbfa1), collagen type1 α1 (Col-1), alkaline phosphatase (ALP), osteopontin (OPN), bone sialoprotein (BSP), osteocalcin (OCN), vitamin D receptor (VDR), cementum protein-1 (CEMP-1), cementum attachment protein (CAP), interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1) and osteoprotegerin (OPG) are mineralization-related modulators. Cbfa1, ALP, OPN, BSP, OCN, VDR, IL-6, TGF-β1 and OPG are transcription factors associated with osteoblast differentiation, formation, bioactivity and bone remodeling.[1] On the other hand, CEMP-1 and CAP are those regulate the formation and regeneration of cementum and surrounding connective tissues.[2,3], All these were used to examine the extent and ability of osteogenesis and cemementogenesis.

Enamel matrix derivative (EMD) includes a group of amelogenins derived from the Hertwig's root sheath of porcine origin. It has been shown that the attachment rate, growth factor production, and metabolism of human periodontal ligament cells in culture were all significantly increased in the presence of EMD.[4,5], Vitamin C (Vit.C) is vital for mesenchymal stem cells differentiation and plays an important role in bone remodeling.[6]

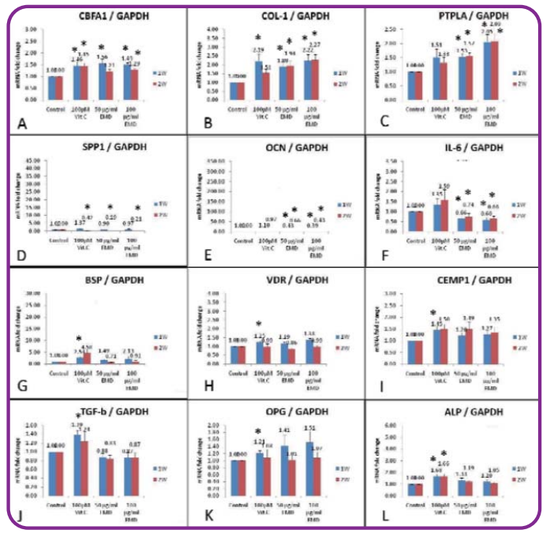
The purpose of this study was to survey the osteoinductive and cementoinductive effects of EMD and Vit.C on mRNA level of the above mentioned biomarkers in human PDL derived cells (hPDCs).

Material and methods

HPDCs derived from six healthy individuals, ages ranging from 18 to 56 who were undergoing premolar extractions for orthodontic reasons or of third molars for malposition extraction. These cells were cultured and then treated with different media. Four groups, categorized as control, 50ug EMD, 100ug EMD, and 10^{-5} M Vit.C groups, were tested. The expressions of mRNA fold-change were assessed by quantitative real-time polymerase chain reaction at 1st and 2nd week after treatment. The significance were analyzed by Wilcoxon singed-rank test at the level of $p<0.05$.

Results

EMD enhanced Cbfa1, Col-1 and CAP (PTPLA: protein tyrosine phosphatase like protein a) mRNAs fold-changes significantly, but decreased OPN (SPP1: secreted phosphoprotein 1), OCN and IL-6 mRNAs than control. (Fig. A-F) Vit.C, as a positive control, functioned on Cbfa-1 and ALP mRNAs expression at two tested time-points, and increased significant Col-1, BSP, VDR, CEMP-1, TGF-β1, and OPG mRNAs at 1st week. (Fig.B,G-K) However, it down-regulated OPN mRNA at 2nd week. (Fig.D) Basically, Vit.C up-regulated more ALP, OPN (SPP1), OCN, CEMP-1, IL-6 and TGF-β1 mRNAs expression than did EMD. (Fig. D-F,I,J,L)



◀ **Figure A-L**
mRNA fold changes of mineralization-related biomarkers receiving treatment with Vit. C and EMD at 1st and 2nd week. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. *Significant difference between control and test group ($p<0.05$).

Discussion

In accordance to previous studies, periodontal ligament derived cells treated with EMD (5-100 μg) resulted in increased mRNA expressions of Col-1, CAP and

Cbfa-1 which represented the potential for EMD to enhance the periodontal regeneration. However, the inconsistence existed in the regulation effect of EMD on mRNA expressions of OPN, OCN and IL-6. It may contribute to the different media of cell culture and the different cell passaging. In Wang's study, all cells were cultured with the supplement of 50 μg/mL vitamin C which in our study revealed to be a positive control on mineralization-related mRNA expression. In addition, Miron found that EMD significantly increased cell proliferation and differentiation of cells at passages between 2 to 5 however had completely lost their ability to respond to EMD by passages more than 10.[7,8]

In our study, we found the positive effect of Vit.C to mineralization in human PDL derived cells which was similar to Ishikawa's result. According to Ishikawa et al., the up-regulation of Col-1 mRNA expression by Vit.C was accompanied with the enhanced expression of α2β1 integrin, a major receptor of type 1 collagen. These results suggested that Vit.C promotes the osteoblastic differentiation of PDL cells by modulating type I collagen-α2β1 integrin interaction.[6]

Conclusion

Generally, Vit.C intensified more fold-changes for the tested osteoinductive and cementoinductive mRNAs. However, EMD up-regulated CAP mRNA in this study.

Reference

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論文原稿

EMD與Vitamin C對牙周韌帶衍生細胞礦化相關mRNA表現的調節

Regulation of mineralization-related mRNA expressions by enamel matrix derivative and vitamin C in human periodontal ligament derived cells

梅至君 洪祥熙
長庚紀念醫院林口醫學中心牙科系牙周病科
Mei, Chih-Chun; Hong, Hsiang-Hsi
Department of Periodontics, Section of Dentistry, Chang Gung Memorial Hospital Linkou Medical Center, and Chang Gung University. Taoyuan, Taiwan

Background

The mRNAs of core-binding factor subunit alpha-1 (Cbfa1) , collagen type1 α1 (Col-1), alkaline phosphatase (ALP), osteopontin (OPN), bone sialoprotein (BSP), osteocalcin (OCN), vitamin D receptor (VDR), cementum protein-1 (CEMP-1), cementum attachment protein (CAP), interleukin-6 (IL-6), transforming growth factor-β1 (TGF-β1) and osteoprotegerin (OPG) are mineralization-related modulators. Cbfa1, ALP, OPN, BSP, OCN, VDR, IL-6, TGF-β1 and OPG are transcription factors associated with osteoblast differentiation, formation, bioactivity and bone remodeling.[1] On the other hand, CEMP-1 and CAP are those regulate the formation and regeneration of cementum and surrounding connective tissues.[2,3] All these were used to examine the extent and ability of osteogenesis and cementogenesis.

Enamel matrix derivative (EMD) includes a group of amelogenins derived from the Hertwig`s root sheath of porcine origin. It has been shown that the attachment rate, growth factor production, and metabolism of human periodontal ligament cells in culture were all significantly increased in the presence of EMD.[4,5] Vitamin C (Vit.C) is vital for mesenchymal stem cells differentiation and plays an important role in bone remodeling.[6]

The purpose of this study was to survey the osteoinductive and cementoinductive effects of EMD and Vit.C on mRNA level of the above mentioned biomarkers in human PDL derived cells (hPDCs).

Material and methods

HPDCs derived from six healthy individuals, ages ranging from 18 to 56 who were undergoing premolar extractions for orthodontic reasons or of third molars for malposition extraction. These cells were cultured and then treated with different media. Four groups, categorized as control, 50μg EMD, 100μg EMD, and 10^{-5}M Vit.C groups, were tested. The expressions of mRNA fold-change were assessed by quantitative real-time polymerase chain reaction at 1st and 2nd week after treatment. The significance were analyzed by Wilcoxon singed-rank test at the level of $p<0.05$.

Results

EMD enhanced Cbfa1, Col-1 and CAP (PTPLA: protein tyrosine phosphatase like protein a) mRNAs fold-changes significantly, but decreased OPN (SPP1: secreted phosphoprotein 1), OCN and IL-6 mRNAs than control. (Fig.A-F) Vit.C, as a positive control, functioned on Cbfa-1 and ALP mRNAs expression at two tested time-points, and increased significant Col-1, BSP, VDR, CEMP-1, TGF-β1, and OPG mRNAs at 1st week. (Fig.B,G-K) However, it down-regulated OPN mRNA at 2nd week. (Fig.D) Basically, Vit.C up-regulated more ALP, OPN (SPP1), OCN, CEMP-1, IL-6 and TGF-β1 mRNAs expression than did EMD. (Fig. D-F,I,J,L)

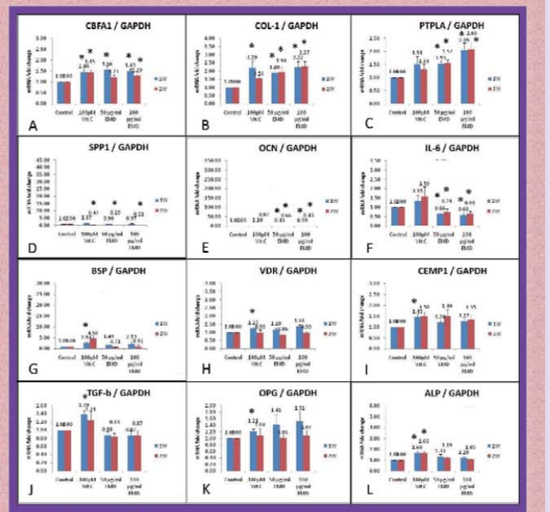

Figure A-L. mRNA fold changes of mineralization-related biomarkers receiving treatment with Vit. C and EMD at 1st and 2nd week.
GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
*Significant difference between control and test group ($p<0.05$).

Discussion

In accordance to previous studies, periodontal ligament derived cells treated with EMD (5-100μg) resulted in increased mRNA expressions of Col-1, CAP and Cbfa-1 which represented the potential for EMD to enhance the periodontal regeneration. However, the inconsistence existed in the regulation effect of EMD on mRNA expressions of OPN, OCN and IL-6. It may contribute to the different media of cell culture and the different cell passaging. In Wang's study, all cells were cultured with the supplement of 50 μg /mL vitamin C which in our study revealed to be a positive control on mineralization-related mRNA expression. In addition, Miron found that EMD significantly increased cell proliferation and differentiation of cells at passages between 2 to 5 however had completely lost their ability to respond to EMD by passages more than 10.[7,8]

In our study, we found the positive effect of Vit.C to mineralization in human PDL derived cells which was similar to Ishikawa's result. According to Ishikawa et al., the up-regulation of Col-1 mRNA expression by Vit.C was accompanied with the enhanced expression of α2β1 integrin, a major receptor of type 1 collagen. These results suggested that Vit.C promotes the osteoblastic differentiation of PDL cells by modulating type I collagen-α2β1 integrin interaction.[6]

Conclusion

Generally, Vit.C intensified more fold-changes for the tested osteoinductive and cementoinductive mRNAs. However, EMD up-regulated CAP mRNA in this study.

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壁報論文作品欣賞 醫院組

第三名

Prediction of the Need for Orthognathic Surgery in Teenagers with Unilateral Cleft Lip and Palate Using Receiver Operating Characteristic Analysis

Yun-Chia Ku, Wen-Ching Ko
Department of Craniofacial Orthodontics, Chang Gung Memorial Hospital

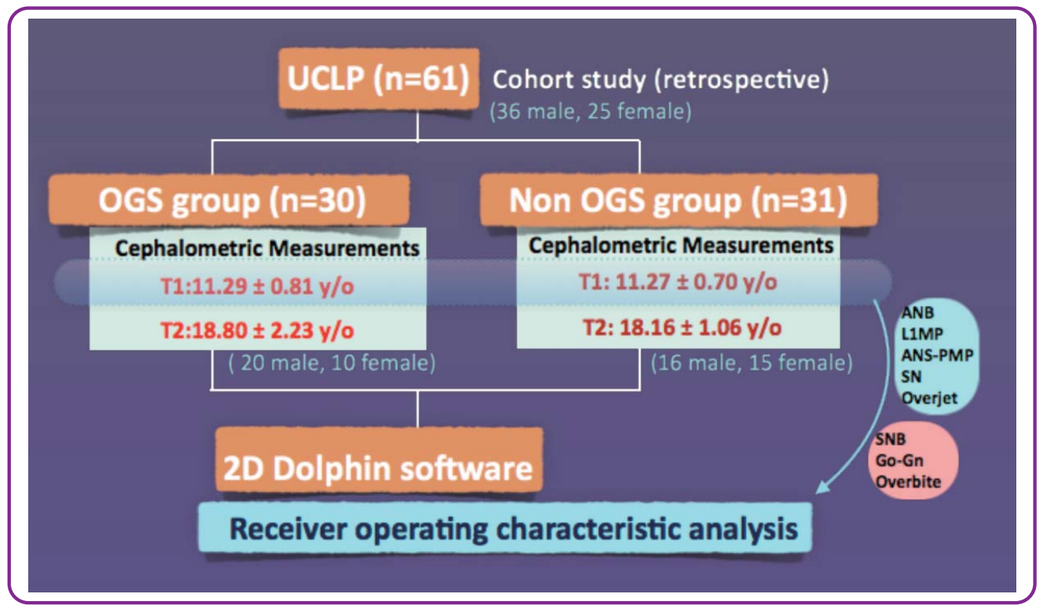
長庚紀念醫院 - 古芸家、柯雯青 醫師

Introduction

Unilateral cleft lip and palate (UCLP) is one of the most common craniofacial anomalies involving the failure of facial tissues to join properly during development. Ross et al. suggested that growth deficiency in individuals with UCLP can be attributed to the intrinsic factor being developmental deficiency and the iatrogenic factor being the influence of the surgical repair of the lip and palate. Studies have reported a need for orthognathic surgical (OGS) correction in a frequency varying from 12.5% to 48%. Our goal was to develop a scoring system to predict a future need for OGS based on cephalometric variables at the age of 11 years for individuals with UCLP.

Material

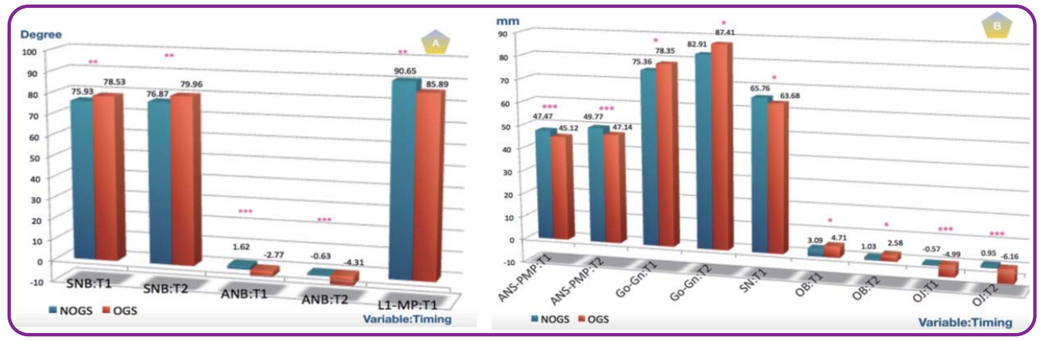
In this retrospective cohort study, 61 Taiwanese individuals with complete UCLP were investigated. All individuals had been treated with the same protocol from infancy to adolescence and were under observation until the end of the growth period in our center. Individuals who had incomplete clefts, associated anomalies were excluded. Individuals were divided into the OGS (n=30) and the non-OGS (NOGS) groups (n=31) according to the hospital records at the mean age of 18.5 years. The orthodontist decided the need for OGS. Lateral cephalograms of each individual were taken with rulers and were analyzed at an age of approximately 11 years (T1) and at the completion of growth before receiving an OGS (T2). Receiver operating characteristic (ROC) analysis was performed to determine the ability of cephalometric measurements to distinguish between the 2 groups at the age of 11 years.



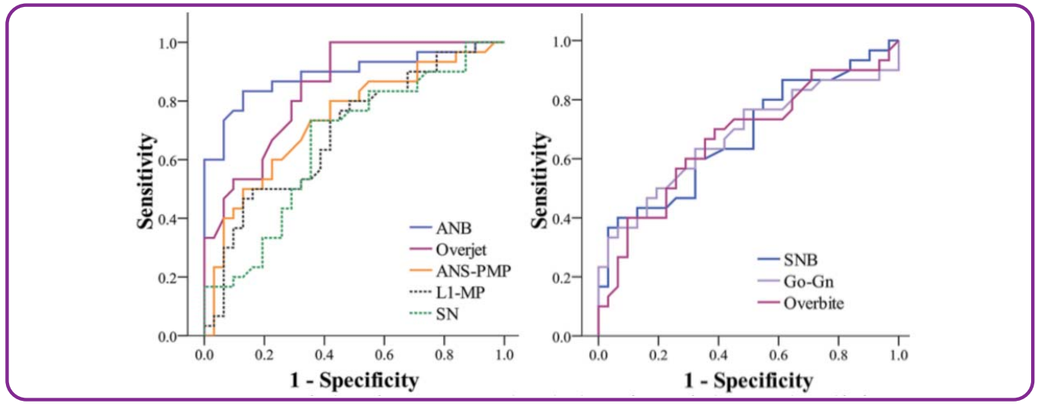
▲UCLP說明圖

Results

The distribution of gender (p=0.23) and the cleft side (p=0.28) were not significantly differ between the 2 groups. Significant differences were observed for 8 variables (SNB, ANB, SN, overbite, overjet, maxillary length, mandibular body length, and L1-MP) between the OGS and the NOGS groups at the age of 11 years (Figure 1). These variables were used to identify the need for OGS by using receiver operator characteristic (ROC) curves (Figure 2). The area under the



▲FIGURE 1
Cephalometric variables with significant difference between the OGS and the NOGS group at each time point.



▲Figure 2
ROC curves of eight significant measurements obtained at the age of 11 years for determining the need for future OGS.

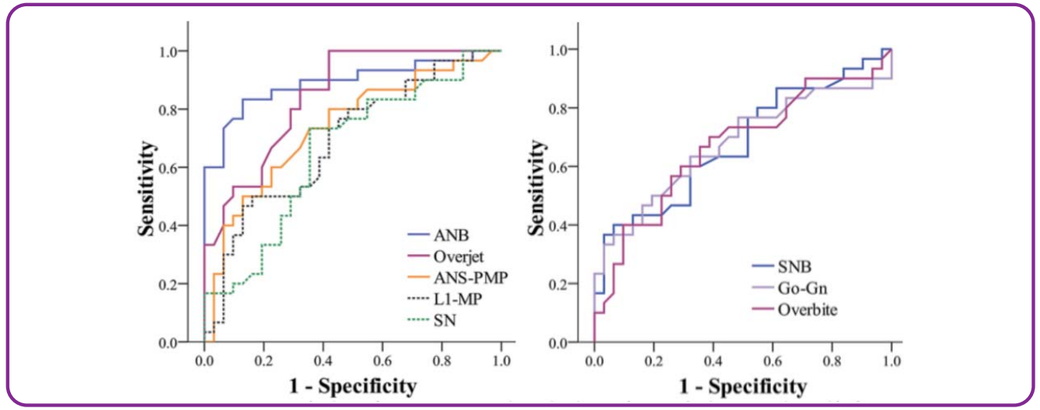
curve (AUC) of each variable was determined. The corresponding cutoff point with the maximum sum of sensitivity and specificity was determined for each variable (Table 1). The 8 cephalometric variables were dichotomized into 2 parts based on their cutoff points and were transferred into a new score of 1 and 0. On the basis ofclinical criteria, the part with a tendency for OGS was scored as 1 and the other part was scored as 0. The 8 dichotomized variables were added one by one from the variables with the highest to lowest AUC values to create 8 different scoring systems (Table 2, Figure 3). The AUC values of these scoring systems were calculated to determine the optimal number of dichotomized variables for inclusion in the final scoring system. A scoring system model based on 3 dichotomized variables (ANB, <-0.45°; overjet, <-2.00 mm; and maxillary length, <47.25 mm) yielded the highest AUC (0.893) and the best diagnostic accuracy of 86.9%. Hence, for this scoring system, the possible scores of 0 to 3 corresponded to 3 variables with values within the part of score 1 (tendency for OGS). Table 3 lists the sensitivity and specificity of scores 0 to 3. A score of 2 indicated the most favorable combination of sensitivity (90.0%) and specificity (83.9%) for predicting the requirement for surgical treatment at the end of growth. Individuals receiving scores of 2 and 3 in the 3-variable based scoring system had the probabilities of 62.6% and 91.8% need for future OGS. The obtained results were in favor of the hypothesis that it is possible to identify individuals with UCLP that are candidates for OGS at age of 11 years.

Variable	AUC	Cutoff point	Score 1	Score 0	Sensitivity	Specificity
ANB	0.891	-0.45 °	≤-0.45 °	> -0.45 °	0.833	0.844
Overjet	0.846	-2 mm	≤ -2 mm	> -2 mm	1	0.594
ANS-PMP	0.737	47.25 mm	≤47.25 mm	> 47.25 mm	0.8	0.562
L1-MP	0.691	91.2 °	≤ 91.2 °	> 91.2 °	0.8	0.5
SNB	0.678	77.55 °	≥ 77.55 °	< 77.55 °	0.6	0.656
Go-Gn	0.676	76.7 mm	≥76.7 mm	< 76.7 mm	0.633	0.687
Overbite	0.670	3.25 mm	≥ 3.25 mm	< 3.25 mm	0.7	0.594
SN	0.659	65.25 mm	≤ 65.25 mm	> 65.25 mm	0.733	0.625

▲TABLE 1
Baseline characteristics of the collected deciduous dental pulps (N = 161)

Number of variables	Range of Score	Variables	AUC	Accuracy %
1 variable	0~1	ANB	0.849	85.2
2 variables	0~2	Above measurement plus overjet	0.876	80.3
3 variables	0~3	Above measurements plus ANS-PMP	0.893	86.9
4 variables	0~4	Above measurements plus L1-Mp	0.885	85.2
5 variables	0~5	Above measurements plus SNB	0.888	82
6 variables	0~6	Above measurements plus Go-Gn	0.885	80.3
7 variables	0~7	Above measurements plus overbite	0.877	75.4
8 variables	0~8	Above measurements plus SN	0.890	78.7

▲TABLE 2
Eight scoring systems of prediction for OGS in the cumulated top-ranked cephalometric measurements (cumulative scores).



▲Figure 3
ROC curves of 8 different scoring systems for determining the need for future OGS.

Number of variables	Probability for requiring OGS	Sensitivity	Specificity	Sensitivity + Specificity	True Positive	True Negative	False Positive	False Negative
3	0.91784	0.6	0.935	1.535	18	29	2	12
2	0.62590	0.9	0.839	1.739	27	26	5	3
1	0.20038	0.967	0.355	1.322	29	11	20	1
0	0.03617	1	0	1	30	0	31	0

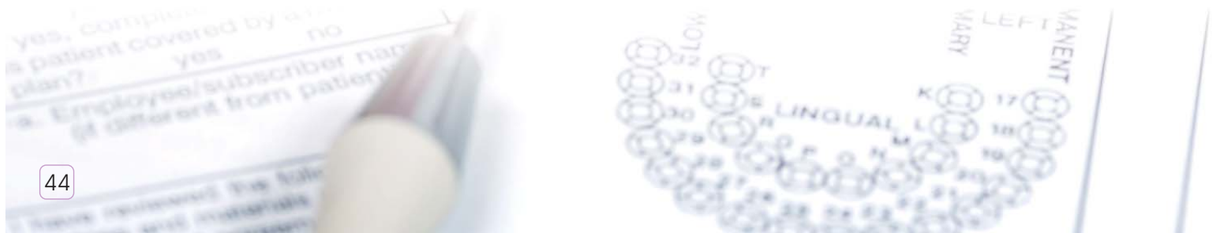
▲TABLE 3
Identifying the cutoff point in scoring system based on 3 dichotomized variables

Discussion

The iatrogenic factor between the OGS and the NOGS groups was decreased with the same protocol for lip and palate repair in our center. The result indicated that the mandible growth had a higher influence on the future need for OGS than did the maxillary growth. There was no significant difference of SNA between the 2 groups was revealed in our research. The ROC analysis can assist in clinical diagnoses using a simplified scoring system to identify poor prognoses in individuals with UCLP at age of 11 years with insensitivity to the distribution of measurements. These 8 variables showed different relative weight for the prediction of growth. Variables with AUC value closer to 0.5 provided less ability to discriminate individuals with OGS needs. A scoring system with a combination of 3 dichotomized variables yielded the highest AUC value and more accurate prediction. However, the AUC value decreased with the inclusion of more dichotomized variables.

Conclusion

- Individuals with UCLP requiring OGS in adulthood had a significantly larger skeletal discrepancy, more mandibular growth, and shorter maxillary length and anterior cranial base at the age of 11 years. ANB, overjet, and maxillary length were identified as the most crucial parameters for identifying the unfavorable prognosis of craniofacial development in individuals with UCLP.
- Three cephalometric variables, which were selected as the minimum number of discriminators required to obtain the optimum discriminant effectiveness, predicted the future need for OGS in individuals with UCLP with an accuracy of 86.9% at the age of 11 years (ANB, <-0.45°; overjet, <-2.00 mm; and maxillary length, <47.25 mm).
- In our 3-variable based scoring system, a score of 2 provided a better prediction of the requirement for surgical treatment, with a sensitivity of 90.0% and a specificity of 83.9%.
- In individuals with UCLP with a possible need for OGS at the completion of growth,aligning the occlusion in early permanent dentition should be deferred.



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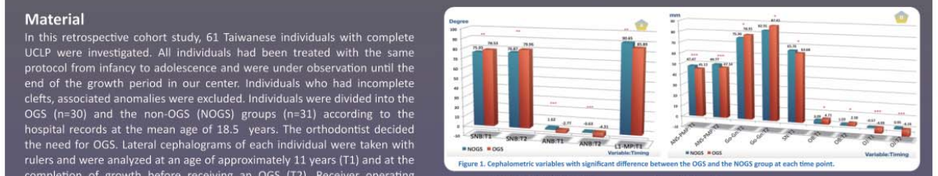

Figure 1. Cephalometric variables with significant difference between the OGS and the NOGS group at each time point.

Table 1. Eight AUC of eight significant measurements from T1 time point.

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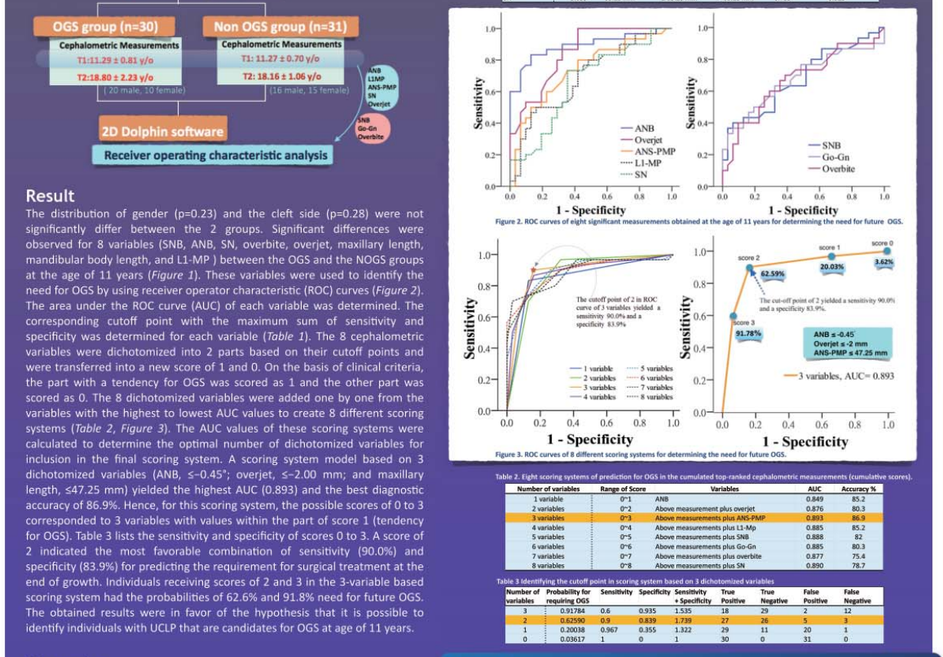

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