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Establishment and pilot identification of affinity purification for human telomerase

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Abstract **Objective**: To establish a method for affinity purification of human telomerase. **Methods**: A fragment of human telomwrase RNA (hTRcDNA) was purified and collected with electroelution after the fragment was identified with restriction enzyme digestion. The affinity chromatography beads were prepared with binding of biotinylated hTRcDNA fragment with streptavidin agarose beads. The purification was based on the ribonucleoprotein property of telomerase with hTRcDNA complementary to hTR template component. After binding reaction between the cancer cell extract with high telomerase activity and the affinity chromatography beads was set up, a complex of the affinity chromatography bead-telomerase was formed. Bound telomerase was eluted with nucleic acid denaturation reagent. Telomerase activity was detected with telomerase PCR-ELISA. The quantity of protein and nucleic acid was assayed with Beckman DU-640 spectrophotometer. **Results**: The result of restriction fragment analysis tallied with the structural map of hTR plasmid vector. The binding quantity and the rate of biotinylated hTRcDNA with streptavidin agarose beads were 31.71 μg and 98.38% respectively. The affinity-purified telomerase kept active and its relative purity was 108.46. **Conclusion**: Our purification method provides a feasible tool for further studies of human telomerase.

Key words telomerase; ribonucleoprotein; affinity purification; gastric neoplasm

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方丝弓矫治技术结合上颌前牙平面导板矫治重度深覆𬌗

Treatment of deep overbite by using edgewise appliances in conjunction with a guiding-plate

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在正畸的临床工作中,对前牙深覆𬌗矫治成功的关键取决于咬合的打开及颌间关系的调整,作者采用上颌前牙的平面导板结合 Edgewise 技术联合矫治,收到满意的临床效果。

1 临床资料与方法

前牙深覆𬌗Ⅱ度35例,男15例,女20例,平均年龄14.5岁,其中15岁以下20例,伴后牙锁𬌗者6例;Angle Ⅰ类7例,Angle Ⅱ类28例。

35例错𬌗根据牙齿拥挤度及𬌗关系调整的需求,除5例不拔牙者,其余均拔除$\frac{4}{4}\frac{4}{4}$。35例均进行X线头影测量分析,测量治疗前后覆𬌗情况。

小平导的制作:$\frac{2\,1|1\,2}{}$间或$\frac{6\,5|5\,6}{}$间作邻间沟,$\frac{3|3}{}$的腭侧形成平面导板,其高度使后牙𬌗间距打开1～2mm,嘱患者全天戴用,直到打开咬合。

矫治步骤:对35例患者,先用Ni-Ti丝排齐牙列,并用摇椅弓打开咬合。待牙列基本排齐后,即戴小平导,戴小平导期间可拉尖牙向远中。对Angle Ⅱ类错𬌗,也可行Ⅱ类牵引,待咬合打开后,即停戴小平导,随后按Edgewise矫治方法进行治疗。

X线头影测量:矫治前后分别拍摄X线头颅侧位片,选择有关测量指标,测量结果采用配对 t 检验。

2 结果

用Edgewise矫治器结合小平导完成矫治的时间为(13.8±4.8)个月,打开咬合的时间为2～4个月。X线头影测量结果,见表1。

固定矫治器结合小平导矫治方法在少年与成年人之间,其打开咬合的量值,所需时间及速度差异均无显著差异。

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Cloning, expression and mutation of mitochondrial ATPase gene of hypertensive patients and rats

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Abstract Objective: To study the different expression of mitochondrial ATPase gene in normotensive and hypertensive human beings and rats. Methods: Partial cDNA clones were screened from human heart cDNA library with differential hybridization. Several mitochondrial genes were highly expressed in the DNA of the cardiac muscle of rats with spontaneous hypertension (SHRs) and in that of peripheral blood leukocytes of patients with hypertension. PCR-SSCP and sequencing were used to study the mutation of the genes. Results: There was polymorphism of mitochondrial ATPase 6 gene in the myocardium of SHR. The base of 8701 changed from G to A (G8701A) and the coden of 59 changed from alanine to threonine and the base of 8708 from A to G and the coden of 61 from histidine to arginine. The mitochondrial ATPase 6 gene in the leucocytes of hepertensive patients underwent similar mutations of G8584A and from alanine to threonine in coden 20. Conclusion: The mutation of G8584A in hypertensive patients is a specific change in hypertension and! may be responsible for the initiation and development of myocardial hypertrophy.

Key words hypertension; mitochondrium; ATPase 6 gene; cloning; mutation

（上接 728 页）

表 1　35 例治疗前后 X 线头影测量结果

项目	治疗前	治疗后	
SNA°	84.14	83.23	——
SNB°	78.2	80.5	$P<0.05$
ANB°	5.8	2.43	$P<0.01$
Yaxis°	66.32	66.95	——
N-ANS	50.4	54.5	$P<0.05$
ANS-Me	51.7	59.2	$P<0.05$
U_1-L_1°	90.4	111.3	$P<0.01$
U_1-SN°	60.2	70.4	$P<0.01$
U_1-NA	12.3	6.5	$P<0.01$
L_1-NB	11.75	8.02	$P<0.01$
L_1-NB°	40.08	34.45	$P<0.01$
L_1-G_0G_n°	62.3	90.6	$P<0.01$

3　讨论

深覆𬌗的形成主要是前牙牙槽骨高度过大而后牙牙槽骨高度不足引起的[1]。打开咬合的机制是：①压低切牙，阻止切牙的萌出。②升高后牙。固定矫治器矫治深覆𬌗是以压低切牙为主，而小平导可使后牙牙槽骨高度明显增加，两者作用互补，比单纯用一种方法打开咬合所需时间短。其次，用小平导使原来无法安放的颊面管和托槽在矫治开始时就可以粘合，避免了因𬌗龈距离过短，或咬合过紧，使颊面管和弓丝损坏，托槽反复脱落而影响咬合打开。总之，用小平导与摇椅弓协同作用，从根本上改善 Spee 氏曲线及补偿曲线，较快打开咬合，及早改善面型。

固定矫治器结合小平导适用于：①后牙牙槽高度不足，面下 1/3 较短者；②前牙覆𬌗过深，咬合过紧，无法粘合托槽；③使用固定矫治器打开咬合，疗效不明显者。固定矫治器结合小平导对年龄较大的患者同样有效。一般认为，年龄较大者生长发育已停止，后牙不可能升高。本研究结果显示，成年组和少年组在打开咬合的总量值，所需时间方面均无显著性差异。伴有后牙锁𬌗者，固定矫治器结合小平导能使锁𬌗解除，使疗程缩短。

关键词　方丝弓；矫治技术；深覆𬌗；平面导板
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