PO Box 2345 Beijing 100023, China
Fax: +86-10-85381893
Email: wcjd@wjgnet.com www.wjgnet.com

World Chin J Digestol 2005 September 15;13(17):2128-2137
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R

• 文献综述 REVIEW •

SNP的研究现状及在MMPs研究中的应用

葛金梅, 张忠英, 彭宣宪, 任建林

葛金梅，彭宣宪，厦门大学生命科学院 福建省厦门市 361005
张忠英，厦门市临床检验中心 福建省厦门市 361004
任建林，厦门大学中山医院消化内科 厦门市消化病研究所 福建省厦门市 361004
通讯作者：张忠英，361004，福建省厦门市湖滨南路201号，厦门市临床检验中心. zhangzy@yahoo.com.cn
电话：0592-8778328
收稿日期：2005-08-03 接受日期：2005-08-26

摘要

对单核苷酸多态性(single nucleotide polymorphisms，SNPs)的研究分析近几年被广泛应用于生物及医学研究的诸多领域，筛查SNPs的方法很多，各具特色，并一直不断地发展.本文对筛查SNP的几种常用及最新方法做一简要介绍，其中包括PCR-RFLP，分子信标等.细胞外基质的降解和改变是肿瘤转移的基本条件，基质金属蛋白酶(matrix metalloproteinases，MMPs)是一类依赖锌离子的蛋白水解酶，可以降解细胞外基质、基底膜、以及间质基质，在肿瘤转移中具有重要作用.有些MMP基因序列存在单基因多态性即SNP现象，且最终影响MMP蛋白的功能.对MMPs的单基因多态性研究为进一步从分子水平研究MMPs的结构和功能及MMPs与肿瘤转移的关系提供了一个新的方向.

关键词：肿瘤转移； SNPs； MMPs

葛金梅，张忠英，彭宣宪，任建林. SNP的研究现状及在MMPs研究中的应用. 世界华人消化杂志 2005;13(17):2128-2137
http://www.wjgnet.com/1009-3079/13/2128.asp

0 引言

单核苷酸多态性(single nucleotide polymorphisms，SNPs)是第三代遗传诊断标记，近几年被广泛应用于生物以及医学研究的诸多领域，筛查SNP的方法自从1996年SNP被正式定为第三代遗传标记以来得到很大的发展，进一步促进了对SNP的研究.尤其是芯片技术的应用使SNP研究进入一个新的阶段.基质金属蛋白酶(matrix Metalloproteinases，MMPs)在肿瘤转移中具有重要作用，对MMPs的研究可以揭示肿瘤转移的原因以及机制，有些MMP基因序列存在SNP现象，并与肿瘤转移有密切联系.研究MMPs的SNP为进一步从分子水平探讨MMPs的作用开辟了一条新的途径.

1 单基因多态性

单核苷酸多态性即SNPs是指基因组DNA序列中由于单个核苷酸(A，G，C，T)替换而引起的多态性，它是一种单核苷酸的变异，是继限制性酶切片断长度多态性即RFLP(restriction fragment length polymorphism)以及可变数串联重复序列即VNTR(variable number of tandem repeat)和微卫星多态性(microsatellite polymorphism)之后的又一新一代多态性遗传标记，自从1994年第一次被提出之后，它渐渐成为与分子标记有关各领域研究的焦点[1-24].

作为第三代遗传标记，SNPs在基因组中具有高密度和高保守的特点，人类30亿个碱基中每千个碱基出现一次，初步估计在整个基因组共有300万以上的SNPs.大多数SNPs位于基因组的非编码区，并且有些位于基因组编码区的SNPs所致编码序列的改变并不影响翻译后的氨基酸序列，这种SNPs对个体的表现型是无影响的.但是有的SNPs位于基因启动子中，导致基因转录活性的上升或下降，造成该蛋白的表达量上升或下降，进一步影响其生物学活性.有些位于蛋白质编码区的SNPs可能影响翻译后关键的功能基团的氨基酸序列，从而影响蛋白质的功能，最终导致对特定环境或病因的反应敏感性.目前很多机构都在检测SNP，做SNP图，建立SNP与各种疾病之间的联系，如果得出某些SNP或某些SNP的特定组合与特定疾病、特定地区发病人群乃至个别患者有明显相关性，疾病的诊断和治疗将可以更有针对性，甚至做到个体化.近几年来，SNP筛查在遗传病的研究，药学的应用研究，以及肿瘤研究中都得到应用.

2 SNP的筛查方法

自从SNP受到重视以来，人们对SNP的筛查方法进行了许多探索和改进.传统的方法有单链构象多态性分析(single-strand conformation polymorphism，SSCP)[25-37]、[38-41]等.比较新兴的方法包括Taqman探针技术[42-50]、焦磷酸测序(pyrosequencing)[51-63]、DNA芯片(DNA chip)[64-73]分析、变性高效液相色谱(denaturing high performance liquid chromatography，DHPLC)[74-88]、能量转移标记的等位特异PCR[89]、基质辅助激光解吸附电离飞行时间质谱(matrix assisted laser desorption ionization time of flight mass spectrometry，MALDI-TOF)[72,90-113]等.下面仅介绍几种常用的SNP筛查方法.

2.1 PCR-RFLP方法[38-41] 利用限制性内切酶的酶切位点的特异性，用两种或两种以上的限制性内切酶作用于同一DNA片断，如果存在SNP位点，酶切片断的长度和数量则会出现差异，根据电泳的结果就可以判断是否有SNP

位点以及出现的碱基替换的类型. 该技术应用的前提是SNP的位点必须含有该限制内切酶的识别位点，它是SNP筛查中最经典的方法之一.

2.2 分子信标(molecular beacons)法[114-117] 分子信标(molecular beacons)法由Tyagi *et al*[114]于1998年建立，作者构建了4种分子U型探针，其核苷酸序列除中央位点处分别为T、C、A、G外完全相同，探针的5′端分别用四种荧光物质标记:香豆素(coumarin，发蓝光)-T，荧光素(fluorescein，发绿光)-C，4甲基蕊香红(tetrame thylrhodamine，发桔红色光)-A，德州红(Texas red，发红光)-G，探针的3′端均结合4-[4′-二甲基胺基苯基氮]安息香酸(DABCYL，可淬灭很多荧光物质发出的荧光，可作为一种常用的淬灭物质)，将这4种探针分别与四种模板链(中央位点处分别为A、G、T、C)互补配对结合. 未结合时探针均不发荧光(通过荧光共振能量传递作用)，只有探针与模板链完全互补配对时构象才会由U型变为直线型，从而发出大量荧光，即便只存在一个碱基的错配也不会发出荧光. 可以通过荧光的颜色不同，识别出该位点的碱基种类.

2.3 Taqman荧光探针法[42-50] Taqman荧光探针法的原理是在PCR反应中，将一对荧光染料和荧光淬灭物质的染料对分别结合到Taqman探针的两端. 探针未与目标序列结合时，通过荧光共振能量传递作用使荧光染料不发荧光；完全互补配对后，由于Taqman DNA聚合酶具有5′核酸酶活性，可将荧光染料从探针上切下来，其发出的荧光可用荧光计检测. 如果探针与目标序列中存在错配碱基，就会减少探针与目标序列结合的紧密程度及Taqman DNA聚合酶切割荧光染料的活性，也就影响了荧光释放量，从而使碱基突变链与正常链得以区分.

2.4 DHPLC法[74-88] DHPLC即变性高效液相色谱技术是近年来新发展的一种SNP筛查方法，一种自动、快速、高通量的基因突变筛查技术，在与疾病相关的基因突变检测SNP筛查方面得到了推广应用.

其突变检测的基本原理是[6-9]：含有突变位点的PCR扩增产物经变性、逐步降温退火后，将形成同源和异源双链(即一条为突变链，另一条为正常链)两种DNA分子. 在部分变性条件下，发生错配的异源双链DNA更易于解链为单链DNA，与DNAsep柱结合力降低，比同源双链DNA分子更易于被乙腈洗脱下来，从而与同源双链DNA分离. 一般来说，含变异成分的PCR产物将在DHPLC图谱上比PCR非变异产物多1-2个峰型，因而两者可以被鉴别.

该方法有赖于DNA同源双链与异源双链之间物理性质的差异,根据异源双链和同源双链在变性反向高压液相离子柱层析过程中滞留时间不一致而分离.

2.5 PCR和测序结合法 将可能的SNP位点进行特异性PCR扩增，为了增加其特异性和准确性可采用巢式PCR，然后结合DNA测序(直接测序或克隆载体测序)找到SNP存在位点并确定其碱基替换类型.

该方法原理简单，容易掌握，适合对短基因片断的SNP筛查，因此，仍然被许多科研工作者应用，最新发表的科研文献中有很多是用该方法进行SNP筛查的.

2.6 PCR-MALDI-MS(PCR-matrix-assisted laser desorption ionization mass spectrometry)法[72,90-113] 生命科学的发展总是与分析技术的进步相关联，基质辅助激光解吸附电离(matrix assistant laser desorption ionization，MALDI)是由两位德国的科学家Franz Hillenkamp和Michael Karas于1988年发明的，并且因此获得了美国质谱协会(ASMS)1997年度杰出贡献奖. 这种技术所具有的高灵敏度和高质量检测范围，使得能在pmol(10^{-12})乃至fmol(10^{-15})水平检测分子量高达几十万的生物大分子，从而开拓了质谱学一个崭新的领域——生物质谱，促使质谱技术在生命科学领域获得广泛应用和发展.

其基本原理是将分析物分散在基质分子(尼古丁酸及其同系物)中并形成晶体. 当用激光(337 nm的氮激光)照射晶体时，由于基质分子吸收辐照光能量，导致能量蓄积并迅速产热，从而使基质晶体升华，导致基质和分析物膨胀并进入气相. 由于MALDI常与TOF连在一起,称为基质辅助激光解吸附飞行时间质谱仪(MALDI-TOF-MS)，俗称飞行质谱. 自发明以来，MALDI-TOF-MS常被应用于蛋白质序列分析，制作肽指纹图谱，测量化合物分子量等，在基因领域的研究有DNA序列测定、DNA点突变、遗传病诊断等. 在SNP筛查中，PCR和质谱技术结合，具有精确，灵敏，高通量的特点.

该方法的缺点是受仪器的限制，费用较高，质谱操作前的纯化技术要求高，否则容易引起误差.

2.7 基因芯片(DNA chip)[64-73] 基因芯片又称DNA芯片(DNA chip)，DNA微集阵列(DNA microarray)等，指采用寡核苷酸原位合成或显微打印手段将大量的DNA片段有序地固定排列在固相支持物如尼龙膜，玻片等表面形成探针阵列，然后与标记的样品进行杂交，通过对杂交信号的检测实现快速、高效、并行的多态信息分析. 利用基因芯片技术筛查SNP是随着近几年芯片技术的快速发展、应用、普及而建立的一种高度并行性、高通量、微型化和自动化的检测手段，应用该方法可以寻找新的SNP位点，并实现SNP位点在基因组中的精确定位.

近几年来SNP的筛查方法取得了很大的进展，但大都以PCR方法为基础，结合电泳技术，或结合荧光、质谱、酶联免疫等方法. 除了我们上述的几种方法外，还有以分子杂交为基础的寡核苷酸连接分析(oligonucleotide ligation assay，OLA)[118-121]，等位基因特异性寡核苷酸探针杂交法(allele-specific oligonucleotide hybridization，ASO)[122-125]，动态等位基因特异性杂交(dynamic allele-specific

hybridization，DASH)[126-129]法，单个碱基延伸标记(single base extension-tag，SBE-Tag)[130]法等，各种方法的应用使检测SNP越来越快速，准确，并且高通量，极大地丰富了现有的SNP库，激发了科学家们寻找SNP的热情.

3 MMPs与肿瘤转移的关系

3.1 肿瘤转移 肿瘤转移是制约临床治疗效果的一种恶性生物学行为[131-136]，是造成肿瘤患者死亡的主要原因.肿瘤转移包括一系列过程，必须多次穿透基底膜(basement membrane，BM)，细胞外基质(extral cell matrix，ECM)等，其中细胞外基质的降解和改变是肿瘤转移和血管生成的基本条件，因为肿瘤细胞必须具备降解细胞外基质，基底膜，甚至间质基质的能力，才能向周围浸润，并向血管、淋巴管及远处转移.其中ECM和BM的降解需要多种基质降解酶即MMPs的协同参与才能完成，因此患者肿瘤组织中MMPs的含量和肿瘤的转移往往呈相关性.

3.2 基质金属蛋白酶 基质金属蛋白酶即MMPs是一类依赖锌离子的蛋白水解酶，迄今为止已经发现了26种.MMPs能够降解细胞外基质和基底膜，参与许多生理和病理过程，是肿瘤浸润转移过程中最重要的调控分子之一，涉及肿瘤浸润和转移，血管的生成，在肿瘤的发展中起关键作用.现已在多种人类肿瘤中检测到MMPs的存在，并显示与肿瘤浸润转移力呈正相关.如早期肝癌和甲状腺癌中有MMP-1的表达，在人类结肠癌中MMP-2，MMP-7和MMP-9均过量表达，在乳腺癌中，已检测到MMP-8，MMP-9和MMP-11并且有望作为肿瘤转移诊断标记.有实验表明，表达MMP-9的乳腺癌肿瘤的浸润和转移发生较早，预后差[137-162].

SNP作为近几年来兴起的第三代分子遗传标记，具有密度大，遗传性稳定的特点.MMPs家族部分成员的启动子已经测序，分子水平的调控研究，尤其是对其启动子SNP的研究已经展开并取得进展，本文的后半部分将对此做一阐述.总之，研究MMPs基因的SNP现象，为进一步从转录水平了解MMPs的作用机制提供了一个很好的方向.

4 MMPs中有关SNP的研究

4.1 MMP-1启动子中SNP研究现状 MMP-1是少数可以降解I型以及III型胶原的酶之一，I型和III型胶原是构成胞外基质的主要成分，与肿瘤细胞的侵袭有密切关系[163-165].MMP-1启动子在－1607存在一个SNP，分别为5’-GAT-3’(1G)和5’-GGAT-3’(2G).Walter *et al*[166]应用PCR-RFLP方法研究了31例黑色素瘤转移病例的MMP-1启动子序列－1607位点1G/2G多态性，发现在11q22.23(MMP-1基因的所在位点)处的杂合性缺失(loss of heterogene，LOH)与2G基因型有关：在12例有LOH的黑色素瘤患者中，83%保留有2G基因型，17%保留有1G基因型.由此推测2G基因型与肿瘤的浸润和转移有关.

Zhu *et al*[167]应用PCR-RFLP方法研究MMP-1启动子－1607位点SNP现象发现，2G/2G基因型提高了MMP-1的转录活性，因此2G/2G个体更易患肺癌，尤其在吸烟个体中危险更高.研究还发现：2G/2G个体比1G/1G或1G/2G个体更易提早发展为肺癌.

Nishioka *et al*[168]利用PCR和测序结合法分析了23例子宫颈上皮肿瘤(cervical intraepithelial neoplasias，CIN)标本和86例子宫颈癌(cervical cancer)标本的MMP-1启动子-1607 SNP，并进行统计学分析，发现MMP-1启动子2G SNP和MMP-1的表达之间存在相关性，并和子宫颈癌的临床分期有关.这表明MMP-1启动子的2G SNP可能影响MMP-1基因的转录活性，进而影响子宫颈癌的侵袭和浸润活性.

但也许SNP的作用效果在不同人群中存在差异，据Ju *et al*[45]报道：用TaqMan法对韩国232例子宫颈癌患者血液和332例健康对照个体血液进行MMP-1启动子－1607位点SNP分析发现：2G频率在子宫颈癌患者中为66.1%，而在对照组中为68.2%，二者之间没有明显差异.因此，2G SNP既不导致韩国妇女对子宫颈癌易感，又不促进子宫颈癌的发展.

Matsumura *et al*[169]用PCR-RFLP法分析了215个胃癌患者和166个健康对照个体，发现二者1G/2G的比率接近，并且无论在肿瘤的侵袭程度，淋巴结转移，和临床分期上都无明显的差别.但另一方面，发现SNP和胃癌患者的组织分化和性别分布有着显著关联（$P<0.05$），由此可见，MMP-1的启动子中2G等位基因的存在并不会提高患胃癌的危险，但是可以对胃癌的分化产生影响.

Wyatt *et al*[170]利用PCR和测序结合法测定34例人类包皮成纤维细胞中MMP-1启动子1G/2G多态性和相应MMP-1蛋白表达水平之间的关系，结果表明2G SNP的存在并不会显著提高MMP-1蛋白的含量，但是会提高MMP-1基因对外界刺激因子(如细胞因子，生长因子)的敏感性.此外可以采用PCR和测序结合法发现新的SNP位点，如Jurajda *et al*[171]应用PCR和测序结合法发现了MMP-1启动子中一个新的SNP位点：159A/G，通过对该位点和已经发现的1607 1G/2G的连锁分析发现A等位基因常和－1607的2G等位基因连锁，而G等位基因则常和1G等位基因连锁，从而为以后研究MMP-1表达在肿瘤转移中的意义提供了一个新的方向.

另外，关于MMP-1在转录水平调控机制的研究也早已展开.研究表明MMP-1的表达受有丝分裂素激活蛋白激酶(mitogen-activated protein kinase，MAPK)途径的调控，MAPK途径由三部分组成，分别是胞外信号调控激酶(extracellular signal regulated kinase，ERK)，p38和c-Jun N端激酶(c-Jun N-terminal kinase，JNK).它们都是以核转录因子家族活性蛋白－

1(activated protein-1，AP-1)和ETS转录因子家族为底物的.MMP-1启动子-1607位点的2G SNP提供了一个ETS结合位点，和－1602位点的AP-1结合位点共同作用，促进MMP-1的转录，因此，2G SNP与1G SNP相比，MMP-1的转录活性提高[172, 173].黑色素瘤细胞系A2058是一个2G纯合体，具有高水平的MMP-1组成型表达.Tower *et al*[172]利用Northern blotting，Western blotting，荧光素酶活性分析和PCR为基础的定点突变方法研究发现，如果加入一个针对ERK途径的特异性抑制剂PD098059，则MMP-1的表达受阻，由此可见ERK1/2途径主要以MMP-1的2G多态型为靶点，促进MMP-1基因的转录，从而提高相应肿瘤细胞的转移能力.Tower *et al*[174]再次用乳腺癌细胞MCF-7/ADR研究发现，AP-1位点在2G SNP存在的情况下可以提高MMP-1基因的转录活性，相反在1G SNP存在的情况下则抑制MMP-1基因的转录，从而抑制I型胶原的降解，最终降低了MCF-7/ADR细胞的侵袭能力.可见，2G SNP和ERK1/2途径以及AP-1位点共同协作促进MMP-1的高表达，最终导致乳腺癌细胞的侵袭能力提高.

同样Tower *et al*[175]发现FRA-1(Fos-like region antigen)和AP-1转录因子共同促进A2058中MMP-1蛋白的表达.抑制FRA-1，与1G SNP相比，会明显下调含2G SNP的MMP-1启动子的转录活性.

Zinzindohoue *et al*[176]研究了结肠癌患者的MMP-1启动子2G/2G基因型与患者存活率之间的关系发现，具有2G/2G基因型的各个临床分期患者和同期非2G/2G基因型患者相比较都具有显著较低的存活率，统计分析结果是：I期和II期，$P<0.01$；从I期到III期，$P<0.001$；总体，$P<0.04$.因此，经过临床阶段，年龄，以及化疗辅助各方面因素的校正后，2G SNP可以作为独立的诊断结肠癌不良预后的指标.而同时进行的MMP-3启动子SNP和结肠癌存活率相关性研究则表明，二者无显著相关性.

4.2 MMP-3中SNP研究现状 MMP-3可以降解层黏连蛋白和纤黏连蛋白，对肿瘤发生和肿瘤生长具有重要意义.MMP-3启动子－1171位点存在5A/6A的多态现象.Krippl *et al*[177]应用Taqman荧光标记法对500例已经组织确诊为乳腺癌的患者和500个健康对照个体做了MMP-3启动子的SNP研究，发现5A/5A、5A/6A、6A/6A三种基因型在患者和对照中的分布大致相似，其中患者分别为20.6%、51.8%、27.6%，而对照则分别为23.3%、47.3%、29.4%.但患者中基因型为5A/5A者和5A/6A或6A/6A相比有较高的淋巴结转移率，因此MMP-3启动子的5A/6A多态性不会影响个体对乳腺癌的易感性，但是会提高患者的癌细胞转移能力.

Zhang *et al*[178]用PCR-RFLP方法分析了中国北方417位患者，其中食道鳞细胞癌(esophageal squamous cell carcinoma，ESCC)234例，胃贲门腺癌(gastric cardiac adenocarcinoma，GCA)183例，以及350例健康对照个体的MMP-3启动子的5A/6A SNP，发现ESCC患者中SNP至少含一个5A等位基因的个体具有明显的淋巴转移倾向，而在GCA患者中却没有观察到，因此MMP-3的5A SNP和ESCC的肿瘤发展以及淋巴转移有关.

Fang *et al*[179]为了研究MMP-3启动子5A/6A SNP和基因易感性、非小细胞肺癌(non-small cell lung cancer， NSCLC)以及淋巴结转移之间的关系，利用PCR-RFLP方法分析了173例NSCLC患者和350例健康对照个体的MMP-3启动子SNP，结果是：6A/6A、5A/6A、5A/5A三种基因型在NSCLC患者中的比例分别是65.3%、30.6%、4.1%，在健康个体中的比例分别是67.7%、30.0%、2.3%，可见总体基因型在患者和对照之间并没有明显的差异.但是，5A SNP在吸烟的患者中比在健康吸烟者中更常见.还发现：5A/5A基因型的患者和6A/6A基因型患者相比，具有更大的淋巴结转移的危险.因此，MMP-3 5A等位基因可能与吸烟者中NSCLC的易感性有关，并且5A/5A基因型可能会提高NSCLC患者的淋巴转移.

4.3 MMP-7中的SNP研究现状 众多的研究表明，MMP-7与人的子宫内膜癌以及胃肠消化道癌的肿瘤转移有关.在MMP-7启动子－181有一个A/G SNP位点.Zhang *et al*[180]用PCR-RFLP方法分析了258例ESCC，201例GCA，243例NSCLC，以及350例健康个体的MMP-7启动子A/G SNP现象，发现A/G或G/G SNP明显提高对以上三种癌症的易感性，方差分析表明－181G和GCA以及NSCLC之间有显著相关性，因此MMP-7的－181A/G SNP可以作为GCA和NSCLC的基因易感标记物.

4.4 MMP-9中的SNP研究现状 MMP-9是IV型胶原酶，是可以降解ECM主要骨架蛋白IV型胶原的蛋白水解酶之一，已经成为近几年肿瘤研究的焦点，是极具吸引力的肿瘤治疗的靶标，已经针对它们设计了一些有希望的抗肿瘤药物.

MMP-9启动子－1562存在一个C/T SNP，影响基因的表达，Matsumura *et al*[181]应用PCR-RFLP方法分析了177例胃癌和224例健康个体的MMP-9启动子SNP，结果发现二者的基因型没有明显的差别，但是SNP和肿瘤侵袭、临床阶段、以及淋巴转移有明显的相关性($P<0.05$)，因此MMP-9启动子中T等位基因和胃癌的侵袭性有关.

5 展望

迄今为止，SNP的筛查技术和方法越来越简便，精确，高通量，SNP筛查水平的提高必将为肿瘤的发生、发展、恶化、转移等各阶段的作用机制提供一个更微观的研究方法和思路，现阶段对26种MMPs中的SNP筛查仅着力于其中的一部分，比如MMP-1、MMP-3、MMP-9，并且大都集中研究基因启动子的SNP现象，而对结构基因的SNP筛查和研究尚未见报道.可以预见，随着MMPs对肿瘤转移的作用机制在蛋白质结构水平的研究进展，将会揭

示有关结构基因SNP影响MMPs蛋白功能基团的氨基酸序列，进而影响酶的活性，以及肿瘤转移程度等各方面的信息.另外，现阶段大多从研究MMPs在病理组和正常对照组之间是否有显著差异方面入手，今后可进一步深入研究MMPs的SNP与其他的肿瘤转移因子的相互作用.总之，相信随着研究的进展，随着基因芯片技术的应用以及对人类基因组的日新月异的深入了解，必将发现更多MMPs的SNPs，揭示更多SNPs与肿瘤转移的相关性，为相应肿瘤诊断，预后评估，抗肿瘤药物研究甚至基因诊断和基因治疗提供依据.

6 参考文献

1 Nordborg M, Borevitz JO, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA, Weigel D. The extent of linkage disequilibrium in Arabidopsis thaliana. *Nat Genet* 2002;30:190-193

2 Primmer CR, Borge T, Lindell J, Saetre GP. Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. *Mol Ecol* 2002;11:603-612

3 Rafalski A. Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol* 2002;5:94-100

4 Pritchard JK, Przeworski M. Linkage disequilibrium in humans: models and data. *Am J Hum Genet* 2001;69:1-14

5 Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES 4th. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc Natl Acad Sci USA* 2001;98:11479-11484

6 Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409:928-933

7 See D, Kanazin V, Talbert H, Blake T. Electrophoretic detection of single-nucleotide polymorphisms. *Biotechniques* 2000;28:710-714, 716

8 Stumpf MP. Haplotype diversity and the block structure of linkage disequilibrium. *Trends Genet* 2002;18:226-228

9 Syvanen AC. Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat Rev Genet* 2001;2:930-942

10 Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF, Gaut BS. Patterns of DNA sequence polymorphism along chromosome 1 of maize (Zea mays ssp. mays L.). *Proc Natl Acad Sci USA* 2001;98:9161-9166

11 Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. *Nat Biotechnol* 1996;14:303-308

12 Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 1998;280:1077-1082

13 Wang RL, Stec A, Hey J, Lukens L, Doebley J. The limits of selection during maize domestication. *Nature* 1999;398:236-239

14 Nasu S, Suzuki J, Ohta R, Hasegawa K, Yui R, Kitazawa N, Monna L, Minobe Y. Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa, Oryza rufipogon) and establishment of SNP markers. *DNA Res* 2002;9:163-171

15 Howell WM, Jobs M, Gyllensten U, Brookes AJ. Dynamic allele-specific hybridization. A new method for scoring single nucleotide polymorphisms. *Nat Biotechnol* 1999;17:87-88

16 Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Rafalski AJ. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. *BMC Genet* 2002;3:19

17 Goldstein DB. Islands of linkage disequilibrium. *Nat Genet* 2001;29:109-111

18 Griffin TJ, Hall JG, Prudent JR, Smith LM. Direct genetic analysis by matrix-assisted laser desorption/ionization mass spectrometry. *Proc Natl Acad Sci USA* 1999;96:6301-6306

19 Gut IG. Automation in genotyping of single nucleotide polymorphisms. *Hum Mutat* 2001;17:475-492

20 Hoskins RA, Phan AC, Naeemuddin M, Mapa FA, Ruddy DA, Ryan JJ, Young LM, Wells T, Kopczynski C, Ellis MC. Single nucleotide polymorphism markers for genetic mapping in Drosophila melanogaster. *Genome Res* 2001;11:1100-1113

21 Kruglyak L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nat Genet* 1999;22:139-144

22 Lindblad-Toh K, Winchester E, Daly MJ, Wang DG, Hirschhorn JN, Laviolette JP, Ardlie K, Reich DE, Robinson E, Sklar P, Shah N, Thomas D, Fan JB, Gingeras T, Warrington J, Patil N, Hudson TJ, Lander ES. Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. *Nat Genet* 2000;24:381-386

23 Marth GT, Korf I, Yandell MD, Yeh RT, Gu Z, Zakeri H, Stitziel NO, Hillier L, Kwok PY, Gish WR. A general approach to single-nucleotide polymorphism discovery. *Nat Genet* 1999;23:452-456

24 Mogg R, Batley J, Hanley S, Edwards D, O'Sullivan H, Edwards J. Characterization of the flanking regions of Zea mays microsatellites reveals a large number of useful sequence polymorphisms. *Theor Appl Genet* 2002;105:532-543

25 Mellon I, Hock T, Reid R, Porter PC, States JC. Polymorphisms in the human xeroderma pigmentosum group A gene and their impact on cell survival and nucleotide excision repair. *DNA Repair (Amst)* 2002;1:531-546

26 Chave KJ, Ryan TJ, Chmura SE, Galivan J. Identification of single nucleotide polymorphisms in the human gamma-glutamyl hydrolase gene and characterization of promoter polymorphisms. *Gene* 2003;319:167-175

27 Cho SM, Kim J, Ryu HJ, Kim JJ, Kim HH, Park JH, Kim HT, Kim KH, Cho HY, Oh B, Park C, Kimm K, Jo I, Lee JE, Shin HD, Lee JK. Identification of single nucleotide polymorphisms in the tumor necrosis factor (TNF) and TNF receptor superfamily in the Korean population. *Hum Immunol* 2004;65:710-718

28 Kamio K, Matsushita I, Tanaka G, Ohashi J, Hijikata M, Nakata K, Tokunaga K, Azuma A, Kudoh S, Keicho N. Direct determination of MUC5B promoter haplotypes based on the method of single-strand conformation polymorphism and their statistical estimation. *Genomics* 2004;84:613-622

29 Brym P, Kaminski S, Wojcik E. Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. *J Appl Genet* 2005;46:179-185

30 Maekawa M, Taniguchi T, Uramoto T, Higashi H, Horii T, Takeshita A, Sugimura H, Kanamori M. Pilot study of arbitrarily primed PCR-single stranded DNA conformation polymorphism analysis for screening genetic polymorphisms related to specific phenotypes. *Clin Chim Acta* 2005;355:181-184

31 Bertin I, Zhu JH, Gale MD. SSCP-SNP in pearl millet-a new marker system for comparative genetics. *Theor Appl Genet* 2005;110:1467-1472

32 Park JY, Park MH, Park H, Ha J, Kim SJ, Ahn C. TNF-alpha

and TGF-beta1 gene polymorphisms and renal allograft rejection in Koreans. *Tissue Antigens* 2004;64:660-666
33 Kuwai T, Kitadai Y, Tanaka S, Kuroda T, Ochiumi T, Matsumura S, Oue N, Yasui W, Kaneyasu M, Tanimoto K, Nishiyama M, Chayama K. Single nucleotide polymorphism in the hypoxia-inducible factor-1alpha gene in colorectal carcinoma. *Oncol Rep* 2004;12:1033-1037
34 Ceriotti G, Chessa S, Bolla P, Budelli E, Bianchi L, Duranti E, Caroli A. Single nucleotide polymorphisms in the ovine casein genes detected by polymerase chain reaction-single strand conformation polymorphism. *J Dairy Sci* 2004;87:2606-2613
35 Alvarez-Busto J, Ruiz-Nunez A, Mazon LI, Jugo BM. Detection of polymorphisms in the tumour necrosis factor alpha candidate gene in sheep. *Eur J Immunogenet* 2004;31:155-158
36 Xiao WH, Liu WW. Analysis of methylation and loss of heterozygosity of RUNX3 gene in hepatocellular carcinoma and its clinical significance. *Zhonghua Gan Zang Bing Za Zhi* 2004;12:227-230
37 Kuraoka K, Oue N, Yokozaki H, Kitadai Y, Ito R, Nakayama H, Yasui W. Correlation of a single nucleotide polymorphism in the E-cadherin gene promoter with tumorigenesis and progression of gastric carcinoma in Japan. *Int J Oncol* 2003;23:421-427
38 Tseng LH, Chen PJ, Lin MT, Singleton K, Martin EG, Yen AH, Martin PJ, Hansen JA. Simultaneous genotyping of single nucleotide polymorphisms in the IL-1 gene complex by multiplex polymerase chain reaction-restriction fragment length polymorphism. *J Immunol Methods* 2002;267:151-156
39 Wen AQ, Wang J, Feng K, Zhu PF, Jiang JX. Analysis of polymorphisms in the promoter region of interleukin-1beta by restriction fragment length polymorphism-PCR. *Chin J Traumatol* 2004;7:271-274
40 Zhang XF, Wang YM, Wang R, Wei LZ, Li Y, Guo W, Wang N, Zhang JH. Correlation of E-cadherin Polymorphisms to Esophageal Squamous Cell Carcinoma and Gastric Cardiac Adenocarcinoma. *Ai Zheng* 2005;24:513-519
41 Hamai Y, Matsumura S, Matsusaki K, Kitadai Y, Yoshida K, Yamaguchi Y, Imai K, Nakachi K, Toge T, Yasui W. A single nucleotide polymorphism in the 5' untranslated region of the EGF gene is associated with occurrence and malignant progression of gastric cancer. *Pathobiology* 2005;72:133-138
42 Johnson VJ, Yucesoy B, Luster MI. Genotyping of single nucleotide polymorphisms in cytokine genes using real-time PCR allelic discrimination technology. *Cytokine* 2004;27:135-141
43 Rickert AM, Borodina TA, Kuhn EJ, Lehrach H, Sperling S. Refinement of single-nucleotide polymorphism genotyping methods on human genomic DNA: amplifluor allele-specific polymerase chain reaction versus ligation detection reaction-TaqMan. *Anal Biochem* 2004;330:288-297
44 Saito K, Miyake S, Moriya H, Yamazaki M, Itoh F, Imai K, Kurosawa N, Owada E, Miyamoto A. Detection of the four sequence variations of MDR1 gene using TaqMan MGB probe based real-time PCR and haplotype analysis in healthy Japanese subjects. *Clin Biochem* 2003;36:511-518
45 Ju W, Kang S, Kim JW, Park NH, Song YS, Kang SB, Lee HP. Promoter polymorphism in the matrix metalloproteinase-1 and risk of cervical cancer in Korean women. *Cancer Lett* 2005;217:191-196
46 Gopalraj RK, Zhu H, Kelly JF, Mendiondo M, Pulliam JF, Bennett DA, Estus S. Genetic association of low density lipoprotein receptor and Alzheimer's disease. *Neurobiol Aging* 2005;26:1-7
47 Aoki-Suzuki M, Yamada K, Meerabux J, Iwayama-Shigeno Y, Ohba H, Iwamoto K, Takao H, Toyota T, Suto Y, Nakatani N, Dean B, Nishimura S, Seki K, Kato T, Itohara S, Nishikawa T, Yoshikawa T. A family-based association study and gene expression analyses of netrin-G1 and -G2 genes in schizophrenia. *Biol Psychiatry* 2005;57:382-393
48 Yamada K, Nakamura K, Minabe Y, Iwayama-Shigeno Y, Takao H, Toyota T, Hattori E, Takei N, Sekine Y, Suzuki K, Iwata Y, Miyoshi K, Honda A, Baba K, Katayama T, Tohyama M, Mori N, Yoshikawa T. Association analysis of FEZ1 variants with schizophrenia in Japanese cohorts. *Biol Psychiatry* 2004;56:683-690
49 Isla D, Sarries C, Rosell R, Alonso G, Domine M, Taron M, Lopez-Vivanco G, Camps C, Botia M, Nunez L, Sanchez-Ronco M, Sanchez JJ, Lopez-Brea M, Barneto I, Paredes A, Medina B, Artal A, Lianes P. Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. *Ann Oncol* 2004;15:1194-1203
50 Ren Z, Cai Q, Shu XO, Cai H, Cheng JR, Wen WQ, Gao YT, Zheng W. Genetic polymorphisms in the human growth hormone-1 gene (GH1) and the risk of breast carcinoma. *Cancer* 2004;101:251-257
51 Pettersson M, Bylund M, Alderborn A. Molecular haplotype determination using allele-specific PCR and pyrosequencing technology. *Genomics* 2003;82:390-396
52 Pacey-Miller T, Henry R. Single-nucleotide polymorphism detection in plants using a single-stranded pyrosequencing protocol with a universal biotinylated primer. *Anal Biochem* 2003;317:166-170
53 Wieser F, Fabjani G, Tempfer C, Schneeberger C, Sator M, Huber J, Wenzl R. Analysis of an interleukin-6 gene promoter polymorphism in women with endometriosis by pyrosequencing. *J Soc Gynecol Investig* 2003;10:32-36
54 Tuziak T, Jeong J, Majewski T, Kim MS, Steinberg J, Wang Z, Yoon DS, Kuang TC, Baggerly K, Johnston D, Czerniak B. High-resolution whole-organ mapping with SNPs and its significance to early events of carcinogenesis. *Lab Invest* 2005;85:689-701
55 Garsa AA, McLeod HL, Marsh S. CYP3A4 and CYP3A5 genotyping by Pyrosequencing. *BMC Med Genet* 2005;6:19
56 Meng H, Hager K, Rivkees SA, Gruen JR. Detection of Turner syndrome using high-throughput quantitative genotyping. *J Clin Endocrinol Metab* 2005;90:3419-3422
57 Huang XQ, Roder MS. Development of SNP assays for genotyping the puroindoline b gene for grain hardness in wheat using pyrosequencing. *J Agric Food Chem* 2005;53:2070-2075
58 Alexander AM, Pecoraro C, Styche A, Rudert WA, Benos PV, Ringquist S, Trucco M. SOP3: a web-based tool for selection of oligonucleotide primers for single nucleotide polymorphism analysis by Pyrosequencing. *Biotechniques* 2005;38:87-94
59 Darimont J, Grosch S, Skarke C, Geisslinger G, Lotsch J. Comparison of two screening methods for in-house genotyping in clinical pharmacology units. *Int J Clin Pharmacol Ther* 2005;43:17-22
60 Watters JW, Zhang W, Meucci MA, Hou W, Ma MK, McLeod HL. Analysis of variation in mouse TPMT genotype, expression and activity. *Pharmacogenetics* 2004;14:247-254
61 Nilsson TK, Johansson CA. A novel method for diagnosis of adult hypolactasia by genotyping of the -13910 C/T polymorphism with Pyrosequencing technology. *Scand J Gastroenterol* 2004;39:287-290
62 Ellnebo-Svedlund K, Larsson L, Jonasson J, Magnusson P. Rapid genotyping of the osteoporosis-associated polymorphic transcription factor Sp1 binding site in the COL1A1 gene by pyrosequencing. *Mol Biotechnol* 2004;26:87-90
63 Lavebratt C, Sengul S, Jansson M, Schalling M. Pyrosequencing-based SNP allele frequency estimation in DNA pools. *Hum Mutat* 2004;23:92-97
64 Huber M, Mundlein A, Dornstauder E, Schneeberger C, Tempfer CB, Mueller MW, Schmidt WM. Accessing single nucleotide polymorphisms in genomic DNA by direct multiplex polymerase chain reaction amplification on oligonucleotide microarrays. *Anal Biochem* 2002;303:25-33
65 Sapolsky RJ, Hsie L, Berno A, Ghandour G, Mittmann M, Fan JB. High-throughput polymorphism screening and genotyping with high-density oligonucleotide arrays. *Genet Anal* 1999;14:187-192

66 Kulle B, Schirmer M, Toliat MR, Suk A, Becker C, Tzvetkov MV, Brockmoller J, Bickeboller H, Hasenfuss G, Nurnberg P, Wojnowski L. Application of genomewide SNP arrays for detection of simulated susceptibility loci. *Hum Mutat* 2005;25:557-565

67 Herr A, Grutzmann R, Matthaei A, Artelt J, Schrock E, Rump A, Pilarsky C. High-resolution analysis of chromosomal imbalances using the Affymetrix 10K SNP genotyping chip. *Genomics* 2005;85:392-400

68 Khan AS. Genomics and microarray for detection and diagnostics. *Acta Microbiol Immunol Hung* 2004;51:463-467

69 Russom A, Haasl S, Ohlander A, Mayr T, Brookes AJ, Andersson H, Stemme G. Genotyping by dynamic heating of monolayered beads on a microheated surface. *Electrophoresis* 2004;25:3712-3719

70 Schwonbeck S, Krause-Griep A, Gajovic-Eichelmann N, Ehrentreich-Forster E, Meinl W, Glatt H, Bier FF. Cohort analysis of a single nucleotide polymorphism on DNA chips. *Biosens Bioelectron* 2004;20:956-966

71 Wakai J, Takagi A, Nakayama M, Miya T, Miyahara T, Iwanaga T, Takenaka S, Ikeda Y, Amano M, Urata T. A novel method of identifying genetic mutations using an electrochemical DNA array. *Nucleic Acids Res* 2004;32:e141

72 Nelson MR, Marnellos G, Kammerer S, Hoyal CR, Shi MM, Cantor CR, Braun A. Large-scale validation of single nucleotide polymorphisms in gene regions. *Genome Res* 2004;14:1664-1668

73 Mishiro S. DNA chip, expression profile, and SNP analyses applied for clinical gastroenterology. *Nippon Shokakibyo Gakkai Zasshi* 2004;101:121-126

74 Giordano M, Mellai M, Hoogendoorn B, Momigliano-Richiardi P. Determination of SNP allele frequencies in pooled DNAs by primer extension genotyping and denaturing high-performance liquid chromatography. *J Biochem Biophys Methods* 2001;47:101-110

75 Ezzeldin H, Hoffmayer C, Soong R, Johnson MR, Lee A, Heslin M, Diasio R. Simultaneous detection of variable number tandem repeats, single nucleotide polymorphisms, and allelic imbalance in the thymidylate synthase gene enhancer region using denaturing high-performance liquid chromatography. *Anal Biochem* 2004;334:276-283

76 Coussens AK, van Daal A. Linkage disequilibrium analysis identifies an FGFR1 haplotype-tag SNP associated with normal variation in craniofacial shape. *Genomics* 2005;85:563-573

77 D'Alfonso S, Mellai M, Giordano M, Pastore A, Malferrari G, Naldi P, Repice A, Liguori M, Cannoni S, Milanese C, Caputo D, Savettieri G, Momigliano-Richiardi P. Identification of single nucleotide variations in the coding and regulatory regions of the myelin-associated glycoprotein gene and study of their association with multiple sclerosis. *J Neuroimmunol* 2002;126:196-204

78 Eklund AC, Belchak MM, Lapidos K, Raha-Chowdhury R, Ober C. Polymorphisms in the HLA-linked olfactory receptor genes in the Hutterites. *Hum Immunol* 2000;61:711-717

79 Bagwell AM, Bailly A, Mychaleckyj JC, Freedman BI, Bowden DW. Comparative genomic analysis of the HNF-4alpha transcription factor gene. *Mol Genet Metab* 2004;81:112-121

80 Mellai M, Giordano M, D'Alfonso S, Marchini M, Scorza R, Giovanna Danieli M, Leone M, Ferro I, Liguori M, Trojano M, Ballerini C, Massacesi L, Cannoni S, Bomprezzi R, Momigliano-Richiardi P. Prolactin and prolactin receptor gene polymorphisms in multiple sclerosis and systemic lupus erythematosus. *Hum Immunol* 2003;64:274-284

81 Han W, Lou DH, Wang J, Yip S, Yap M. Application of DHPLC in human genomic SNP screening and genotyping for all-trans-retinol dehydrogenase. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2005;34:248-254

82 Yu B, Sawyer NA, Caramins M, Yuan ZG, Saunderson RB, Pamphlett R, Richmond DR, Jeremy RW, Trent RJ. Denaturing high performance liquid chromatography: high throughput mutation screening in familial hypertrophic cardiomyopathy and SNP genotyping in motor neurone disease. *J Clin Pathol* 2005;58:479-485

83 Nie Q, Lei M, Ouyang J, Zeng H, Yang G, Zhang X. Identification and characterization of single nucleotide polymorphisms in 12 chicken growth-correlated genes by denaturing high performance liquid chromatography. *Genet Sel Evol* 2005;37:339-360

84 Nie Q, Zeng H, Lei M, Ishag NA, Fang M, Sun B, Yang G, Zhang X. Genomic organisation of the chicken ghrelin gene and its single nucleotide polymorphisms detected by denaturing high-performance liquid chromatography. *Br Poult Sci* 2004;45:611-618

85 Abbas A, Lepelley M, Lechevrel M, Sichel F. Assessment of DHPLC usefulness in the genotyping of GSTP1 exon 5 SNP: comparison to the PCR-RFLP method. *J Biochem Biophys Methods* 2004;59:121-126

86 Tournier I, Raux G, Di Fiore F, Marechal I, Leclerc C, Martin C, Wang Q, Buisine MP, Stoppa-Lyonnet D, Olschwang S, Frebourg T, Tosi M. Analysis of the allele-specific expression of the mismatch repair gene MLH1 using a simple DHPLC-Based Method. *Hum Mutat* 2004;23:379-384

87 Schwarz G, Sift A, Wenzel G, Mohler V. DHPLC scoring of a SNP between promoter sequences of HMW glutenin x-type alleles at the Glu-D1 locus in wheat. *J Agric Food Chem* 2003;51:4263-4267

88 Han W, Yip SP, Wang J, Yap MK. Using denaturing HPLC for SNP discovery and genotyping, and establishing the linkage disequilibrium pattern for the all-trans-retinol dehydrogenase (RDH8) gene. *J Hum Genet* 2004;49:16-23

89 Howell WM, Jobs M, Brookes AJ. iFRET: an improved fluorescence system for DNA-melting analysis. *Genome Res* 2002;12:1401-1407

90 Shifman S, Pisante-Shalom A, Yakir B, Darvasi A. Quantitative technologies for allele frequency estimation of SNPs in DNA pools. *Mol Cell Probes* 2002;16:429-434

91 Kim S, Shi S, Bonome T, Ulz ME, Edwards JR, Fodstad H, Russo JJ, Ju J. Multiplex genotyping of the human beta2-adrenergic receptor gene using solid-phase capturable dideoxynucleotides and mass spectrometry. *Anal Biochem* 2003;316:251-258

92 Tost J, Gut IG. Genotyping single nucleotide polymorphisms by MALDI mass spectrometry in clinical applications. *Clin Biochem* 2005;38:335-350

93 Edwards JR, Ruparel H, Ju J. Mass-spectrometry DNA sequencing. *Mutat Res* 2005;573:3-12

94 Jurinke C, Denissenko MF, Oeth P, Ehrich M, van den Boom D, Cantor CR. A single nucleotide polymorphism based approach for the identification and characterization of gene expression modulation using MassARRAY. *Mutat Res* 2005;573:83-95

95 Petkovski E, Keyser-Tracqui C, Hienne R, Ludes B. SNPs and MALDI-TOF MS: tools for DNA typing in forensic paternity testing and anthropology. *J Forensic Sci* 2005;50:535-541

96 Liao HK, Su YN, Kao HY, Hung CC, Wang HT, Chen YJ. Parallel minisequencing followed by multiplex matrix-assisted laser desorption/ionization mass spectrometry assay for beta-thalassemia mutations. *J Hum Genet* 2005;50:139-150

97 Vallone PM, Fahr K, Kostrzewa M. Genotyping SNPs using a UV-photocleavable oligonucleotide in MALDI-TOF MS. *Methods Mol Biol* 2005;297:169-178

98 Sobrino B, Carracedo A. SNP typing in forensic genetics: a review. *Methods Mol Biol* 2005;297:107-126

99 Powell N, Dudley E, M orishita M, Bogdanova T, Tronko M, Thomas G. Single nucleotide polymorphism analysis in the human phosphatase PTPrj gene using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* 2004;18:2249-2254

100 James MR, Hayward NK, Dumenil T, Montgomery GW, Martin NG, Duffy DL. Epidermal growth factor gene (EGF) polymorphism and risk of melanocytic neoplasia. *J Invest Dermatol* 2004;123:760-762

101 Gut IG. DNA analysis by MALDI-TOF mass spectrometry. *Hum Mutat* 2004;23:437-441

102 Lowe CA, Diggle MA, Clarke SC. A single nucleotide polymorphism identification assay for the genotypic characterisation of Neisseria meningitidis using MALDI-TOF mass spectrometry. *Br J Biomed Sci* 2004;61:8-10

103 Stanssens P, Zabeau M, Meersseman G, Remes G, Gansemans Y, Storm N, Hartmer R, Honisch C, Rodi CP, Bocker S, van den Boom D. High-throughput MALDI-TOF discovery of genomic sequence polymorphisms. *Genome Res* 2004;14:126-133

104 Bocker S. SNP and mutation discovery using base-specific cleavage and MALDI-TOF mass spectrometry. *Bioinformatics* 2003;19:i44-53

105 Maksymowych WP, Reeve JP, Reveille JD, Akey JM, Buenviaje H, O'Brien L, Peloso PM, Thomson GT, Jin L, Russell AS. High-throughput single-nucleotide polymorphism analysis of the IL1RN locus in patients with ankylosing spondylitis by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. *Arthritis Rheum* 2003;48:2011-2018

106 Sauer S, Lehrach H, Reinhardt R. MALDI mass spectrometry analysis of single nucleotide polymorphisms by photocleavage and charge-tagging. *Nucleic Acids Res* 2003;31:e63

107 Tost J, Gut IG. Genotyping single nucleotide polymorphisms by mass spectrometry. *Mass Spectrom Rev* 2002;21:388-418

108 Yang H, Wang H, Wang J, Cai Y, Zhou G, He F, Qian X. Multiplex single-nucleotide polymorphism genotyping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Anal Biochem* 2003;314:54-62

109 Sauer S, Gut IG. Genotyping single-nucleotide polymorphisms by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002;782:73-87

110 Pusch W, Wurmbach JH, Thiele H, Kostrzewa M. MALDI-TOF mass spectrometry-based SNP genotyping. *Pharmacogenomics* 2002;3:537-548

111 Nakai K, Habano W, Fujita T, Nakai K, Schnackenberg J, Kawazoe K, Suwabe A, Itoh C. Highly multiplexed genotyping of coronary artery disease-associated SNPs using MALDI-TOF mass spectrometry. *Hum Mutat* 2002;20:133-138

112 Lechner D, Lathrop GM, Gut IG. Large-scale genotyping by mass spectrometry: experience, advances and obstacles. *Curr Opin Chem Biol* 2002;6:31-38

113 Sauer S, Gelfand DH, Boussicault F, Bauer K, Reichert F, Gut IG. Facile method for automated genotyping of single nucleotide polymorphisms by mass spectrometry. *Nucleic Acids Res* 2002;30:e22

114 Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination. *Nat Biotechnol* 1998;16:49-53

115 Mhlanga MM, Malmberg L. Using molecular beacons to detect single-nucleotide polymorphisms with real-time PCR. *Methods* 2001;25:463-471

116 Shi MM. Technologies for individual genotyping: detection of genetic polymorphisms in drug targets and disease genes. *Am J Pharmacogenomics* 2002;2:197-205

117 Lu CW, Chen ZL, Diao ZH, Wang FY. Investigation of single nucleotide polymorphisms in the promoter region of mannan-binding lectin gene in a Han population from Guangdong. *Di Yi Jun Yi Da Xue Xue Bao* 2003;23:1165-1168

118 Genissel A, Pastinen T, Dowell A, Mackay TF, Long AD. No evidence for an association between common nonsynonymous polymorphisms in delta and bristle number variation in natural and laboratory populations of Drosophila melanogaster. *Genetics* 2004;166:291-306

119 Nickerson DA, Taylor SL, Fullerton SM, Weiss KM, Clark AG, Stengard JH, Salomaa V, Boerwinkle E, Sing CF. Sequence diversity and large-scale typing of SNPs in the human apolipoprotein E gene. *Genome Res* 2000;10:1532-1545

120 Iannone MA, Taylor JD, Chen J, Li MS, Rivers P, Slentz-Kesler KA, Weiner MP. Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry. *Cytometry* 2000;39:131-140

121 Delahunty C, Ankener W, Deng Q, Eng J, Nickerson DA. Testing the feasibility of DNA typing for human identification by PCR and an oligonucleotide ligation assay. *Am J Hum Genet* 1996;58:1239-1246

122 Stimpson DI, Knepper SM, Shida M, Obata K, Tajima H. Three-dimensional microarray platform applied to single nucleotide polymorphism analysis. *Biotechnol Bioeng* 2004;87:99-103

123 Azuma H, Obayashi S, Hamasaki H, Koyama T, Aso T. Role of endothelium in the human uterine arteries during normal menstrual cycle. *Br J Pharmacol* 1995;114:902-908

124 Iwasaki H, Ezura Y, Ishida R, Kajita M, Kodaira M, Knight J, Daniel S, Shi M, Emi M. Accuracy of genotyping for single nucleotide polymorphisms by a microarray-based single nucleotide polymorphism typing method involving hybridization of short allele-specific oligonucleotides. *DNA Res* 2002;9:59-62

125 Wen SY, Wang H, Sun OJ, Wang SQ. Rapid detection of the known SNPs of CYP2C9 using oligonucleotide microarray. *World J Gastroenterol* 2003;9:1342-1346

126 Jobs M, Howell WM, Stromqvist L, Mayr T, Brookes AJ. DASH-2: flexible, low-cost, and high-throughput SNP genotyping by dynamic allele-specific hybridization on membrane arrays. *Genome Res* 2003;13:916-924

127 Breen G. Novel and alternate SNP and genetic technologies. *Psychiatr Genet* 2002;12:83-88

128 Prince JA, Brookes AJ. Towards high-throughput genotyping of SNPs by dynamic allele-specific hybridization. *Expert Rev Mol Diagn* 2001;1:352-358

129 Prince JA, Feuk L, Howell WM, Jobs M, Emahazion T, Blennow K, Brookes AJ. Robust and accurate single nucleotide polymorphism genotyping by dynamic allele-specific hybridization (DASH): design criteria and assay validation. *Genome Res* 2001;11:152-162

130 Fan JB, Chen X, Halushka MK, Berno A, Huang X, Ryder T, Lipshutz RJ, Lockhart DJ, Chakravarti A. Parallel genotyping of human SNPs using generic high-density oligonucleotide tag arrays. *Genome Res* 2000;10:853-860

131 Ata N, Oku T, Hattori M, Fujii H, Nakajima M, Saiki I. Inhibition by galloylglucose (GG6-10) of tumor invasion through extracellular matrix and gelatinase-mediated degradation of type IV collagens by metastatic tumor cells. *Oncol Res* 1996;8:503-511

132 Ikeda T, Murakami K, Hayakawa Y, Fujii H, Ohkoshi M, Saiki I. Anti-invasive activity of synthetic serine protease inhibitors and its combined effect with a matrix metalloproteinase inhibitor. *Anticancer Res* 1998;18:4259-4265

133 Kammerer R, Ehret R, von Kleist S. Isolated extracellular matrix-based three-dimensional in vitro models to study orthotopically cancer cell infiltration and invasion. *Eur J Cancer* 1998;34:1950-1957

134 Ikuta M, Podyma KA, Maruyama K, Enomoto S, Yanagishita M. Expression of heparanase in oral cancer cell lines and oral cancer tissues. *Oral Oncol* 2001;37:177-184

135 Rabbani SA, Mazar AP. The role of the plasminogen activation system in angiogenesis and metastasis. *Surg Oncol Clin N Am* 2001;10:393-415

136 Pasco S, Ramont L, Maquart FX, Monboisse JC. Control of melanoma progression by various matrikines from basement membrane macromolecules. *Crit Rev Oncol Hematol* 2004;49:221-233

137 Mira E, Lacalle RA, Buesa JM, de Buitrago GG, Jimenez-Baranda S, Gomez-Mouton C, Martinez AC, Manes S. Secreted MMP9 promotes angiogenesis more efficiently than constitutive active MMP9 bound to the tumor cell surface. *J Cell Sci* 2004;117:1847-1857

138 Matsumoto K, Minamitani T, Orba Y, Sato M, Sawa H, Ariga H. Induction of matrix metalloproteinase-2 by tenascin-X deficiency is mediated through the c-Jun N-terminal kinase and protein tyrosine kinase phosphorylation pathway. *Exp Cell*

Res 2004;297:404-414
139 Cha BY, Park CJ, Lee DG, Lee YC, Kim DW, Kim JD, Seo WG, Kim CH. Inhibitory effect of methanol extract of Euonymus alatus on matrix metalloproteinase-9. *J Ethnopharmacol* 2003;85:163-167
140 Oba K, Konno H, Tanaka T, Baba M, Kamiya K, Ohta M, Kaneko T, Shouji T, Igarashi A, Nakamura S. Prevention of liver metastasis of human colon cancer by selective matrix metalloproteinase inhibitor MMI-166. *Cancer Lett* 2002;175:45-51
141 Okada N, Ishida H, Murata N, Hashimoto D, Seyama Y, Kubota S. Matrix metalloproteinase-2 and -9 in bile as a marker of liver metastasis in colorectal cancer. *Biochem Biophys Res Commun* 2001;288:212-216
142 Suzuki S, Sato M, Senoo H, Ishikawa K. Direct cell-cell interaction enhances pro-MMP-2 production and activation in co-culture of laryngeal cancer cells and fibroblasts: involvement of EMMPRIN and MT1-MMP. *Exp Cell Res* 2004;293:259-266
143 Lopata A, Agresta F, Quinn MA, Smith C, Ostor AG, Salamonsen LA. Detection of endometrial cancer by determination of matrix metalloproteinases in the uterine cavity. *Gynecol Oncol* 2003;90:318-324
144 Vasala K, Paakko P, Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 immunoreactive protein as a prognostic marker in bladder cancer. *Urology* 2003;62:952-957
145 Miyata Y, Kanda S, Nomata K, Hayashida Y, Kanetake H. Expression of metalloproteinase-2, metalloproteinase-9, and tissue inhibitor of metalloproteinase-1 in transitional cell carcinoma of upper urinary tract: correlation with tumor stage and survival. *Urology* 2004;63:602-608
146 Curry TE, Jr., Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev* 2003;24:428-465
147 Stamenkovic I. Matrix metalloproteinases in tumor invasion and metastasis. *Semin Cancer Biol* 2000;10:415-433
148 Sasaki H, Yukiue H, Moiriyama S, Kobayashi Y, Nakashima Y, Kaji M, Kiriyama M, Fukai I, Yamakawa Y, Fujii Y. Clinical significance of matrix metalloproteinase-7 and Ets-1 gene expression in patients with lung cancer. *J Surg Res* 2001;101:242-247
149 Yukiue H, Sasaki H, Kobayashi Y, Nakashima Y, Moriyama S, Yano M, Kaji M, Kiriyama M, Fukai I, Yamakawa Y, Fujii Y. Clinical significance of tissue inhibitor of metalloproteinase and matrix metalloproteinase mRNA expression in thymoma. *J Surg Res* 2003;109:86-91
150 Bloomston M, Shafii A, Zervos EE, Rosemurgy AS. TIMP-1 overexpression in pancreatic cancer attenuates tumor growth, decreases implantation and metastasis, and inhibits angiogenesis. *J Surg Res* 2002;102:39-44
151 Bloomston M, Zervos EE, Rosemurgy AS, 2nd. Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. *Ann Surg Oncol* 2002;9:668-674
152 Hornebeck W, Maquart FX. Proteolyzed matrix as a template for the regulation of tumor progression. *Biomed Pharmacother* 2003;57:223-230
153 Chang C, Werb Z. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends Cell Biol* 2001;11:S37-43
154 Ishihara Y, Nishikawa T, Iijima H, Matsunaga K. Expression of matrix metalloproteinase, tissue inhibitors of metalloproteinase and adhesion molecules in silicotic mice with lung tumor metastasis. *Toxicol Lett* 2003;142:71-75
155 Lengyel E, Schmalfeldt B, Konik E, Spathe K, Harting K, Fenn A, Berger U, Fridman R, Schmitt M, Prechtel D, Kuhn W. Expression of latent matrix metalloproteinase 9 (MMP-9) predicts survival in advanced ovarian cancer. *Gynecol Oncol* 2001;82:291-298
156 Nuttall RK, Pennington CJ, Taplin J, Wheal A, Yong VW, Forsyth PA, Edwards DR. Elevated membrane-type matrix metalloproteinases in gliomas revealed by profiling proteases and inhibitors in human cancer cells. *Mol Cancer Res* 2003;1:333-345
157 Klein G, Vellenga E, Fraaije MW, Kamps WA, de Bont ES. The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. *Crit Rev Oncol Hematol* 2004;50:87-100
158 McKenna GJ, Chen Y, Smith RM, Meneghetti A, Ong C, McMaster R, Scudamore CH, Chung SW. A role for matrix metalloproteinases and tumor host interaction in hepatocellular carcinomas. *Am J Surg* 2002;183:588-594
159 Seiki M, Mori H, Kajita M, Uekita T, Itoh Y. Membrane-type 1 matrix metalloproteinase and cell migration. *Biochem Soc Symp* 2003:253-262
160 Hernandez-Barrantes S, Bernardo M, Toth M, Fridman R. Regulation of membrane type-matrix metalloproteinases. *Semin Cancer Biol* 2002;12:131-138
161 Liu LT, Chang HC, Chiang LC, Hung WC. Histone deacetylase inhibitor up-regulates RECK to inhibit MMP-2 activation and cancer cell invasion. *Cancer Res* 2003;63:3069-3072
162 Sanceau J, Truchet S, Bauvois B. Matrix metalloproteinase-9 silencing by RNA interference triggers the migratory-adhesive switch in Ewing's sarcoma cells. *J Biol Chem* 2003;278:36537-36546
163 Poola I, DeWitty RL, Marshalleck JJ, Bhatnagar R, Abraham J, Leffall LD. Identification of MMP-1 as a putative breast cancer predictive marker by global gene expression analysis. *Nat Med* 2005;11:481-483
164 Kondo S, Wakisaka N, Schell MJ, Horikawa T, Sheen TS, Sato H, Furukawa M, Pagano JS, Yoshizaki T. Epstein-Barr virus latent membrane protein 1 induces the matrix metalloproteinase-1 promoter via an Ets binding site formed by a single nucleotide polymorphism: enhanced susceptibility to nasopharyngeal carcinoma. *Int J Cancer* 2005;115:368-376
165 Lai HC, Chu CM, Lin YW, Chang CC, Nieh S, Yu MH, Chu TY. Matrix metalloproteinase 1 gene polymorphism as a prognostic predictor of invasive cervical cancer. *Gynecol Oncol* 2005;96:314-319
166 Noll WW, Belloni DR, Rutter JL, Storm CA, Schned AR, Titus-Ernstoff L, Ernstoff MS, Brinckerhoff CE. Loss of heterozygosity on chromosome 11q22-23 in melanoma is associated with retention of the insertion polymorphism in the matrix metalloproteinase-1 promoter. *Am J Pathol* 2001;158:691-697
167 Zhu Y, Spitz MR, Lei L, Mills GB, Wu X. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. *Cancer Res* 2001;61:7825-7829
168 Nishioka Y, Sagae S, Nishikawa A, Ishioka S, Kudo R. A relationship between Matrix metalloproteinase-1 (MMP-1) promoter polymorphism and cervical cancer progression. *Cancer Lett* 2003;200:49-55
169 Matsumura S, Oue N, Kitadai Y, Chayama K, Yoshida K, Yamaguchi Y, Toge T, Imai K, Nakachi K, Yasui W. A single nucleotide polymorphism in the MMP-1 promoter is correlated with histological differentiation of gastric cancer. *J Cancer Res Clin Oncol* 2004;130:259-265
170 Wyatt CA, Coon CI, Gibson JJ, Brinckerhoff CE. Potential for the 2G single nucleotide polymorphism in the promoter of matrix metalloproteinase to enhance gene expression in normal stromal cells. *Cancer Res* 2002;62:7200-7202
171 Jurajda M, Muzik J, Izakovicova Holla L, Vacha J. A newly identified single nucleotide polymorphism in the promoter of the matrix metalloproteinase-1 gene. *Mol Cell Probes* 2002;16:63-66
172 Tower GB, Coon CC, Benbow U, Vincenti MP, Brinckerhoff CE. Erk 1/2 differentially regulates the expression from the 1G/2G single nucleotide polymorphism in the MMP-1 promoter in melanoma cells. *Biochim Biophys Acta* 2002;1586:265-274

173 张键，高福禄，刘芝华. ETS因子家族在发育和肿瘤发生中作用的研究进展. 世界华人消化杂志 2003;11:1624-1627
174 Tower GB, Coon CI, Brinckerhoff CE. The 2G single nucleotide polymorphism (SNP) in the MMP-1 promoter contributes to high levels of MMP-1 transcription in MCF-7/ADR breast cancer cells. *Breast Cancer Res Treat* 2003;82:75-82
175 Tower GB, Coon CI, Belguise K, Chalbos D, Brinckerhoff CE. Fra-1 targets the AP-1 site/2G single nucleotide polymorphism (ETS site) in the MMP-1 promoter. *Eur J Biochem* 2003;270:4216-4225
176 Zinzindohoue F, Lecomte T, Ferraz JM, Houllier AM, Cugnenc PH, Berger A, Blons H, Laurent-Puig P. Prognostic significance of MMP-1 and MMP-3 functional promoter polymorphisms in colorectal cancer. *Clin Cancer Res* 2005;11:594-599
177 Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Koppel H, Leithner A, Wascher TC, Paulweber B, Samonigg H. The 5A/6A polymorphism of the matrix metalloproteinase 3 gene promoter and breast cancer. *Clin Cancer Res* 2004;10:3518-3520
178 Zhang J, Jin X, Fang S, Li Y, Wang R, Guo W, Wang N, Wang Y, Wen D, Wei L, Kuang G, Dong Z. The functional SNP in the matrix metalloproteinase-3 promoter modifies susceptibility and lymphatic metastasis in esophageal squamous cell carcinoma but not in gastric cardiac adenocarcinoma. *Carcinogenesis* 2004;25:2519-2524
179 Fang SM, Jin X, Li Y, Wang R, Guo W, Wang N, Zhang JH. Correlation of matrix metalloproteinase-3 polymorphism to genetic susceptibility and lymph node metastasis of non-small cell lung cancer. *Ai Zheng* 2005;24:305-310
180 Zhang J, Jin X, Fang S, Wang R, Li Y, Wang N, Guo W, Wang Y, Wen D, Wei L, Dong Z, Kuang G. The functional polymorphism in the matrix metalloproteinase-7 promoter increases susceptibility to esophageal squamous cell carcinoma, gastric cardiac adenocarcinoma and non-small cell lung carcinoma. *Carcinogenesis* 2005;26:1748-1753
181 Matsumura S, Oue N, Nakayama H, Kitadai Y, Yoshida K, Yamaguchi Y, Imai K, Nakachi K, Matsusaki K, Chayama K, Yasui W. A single nucleotide polymorphism in the MMP-9 promoter affects tumor progression and invasive phenotype of gastric cancer. *J Cancer Res Clin Oncol* 2005;131:19-25

编辑 张海宁

ISSN 1009-3079 CN 14-1260/R 2005 年版权归世界胃肠病学杂志社

• 消息 •

国际肝胆胰协会中国分会第二届全国学术研讨会暨第三届全国普通外科主任论坛第一轮通知

本刊讯 第二届国际肝胆胰协会中国分会学术会议将于 2006-11 月底或 12 月初在武汉举行.

在各方面的大力支持下，国际肝胆胰协会中国分会第一届学术研讨会已于 2004-12 在武汉成功举办，与会代表一千余人，中国人大副委员长吴阶平院士、国际肝胆胰前主席刘允怡教授、Jim Tooli 教授，国际肝胆胰协会侯任主席 Büechler 教授和欧洲肝胆胰协会主席 Broelsch 教授等亲自到会. 会议受到国内外专家及到会代表的一致赞赏，并受到国际肝胆胰协会的通报好评， 会议取得巨大成功.

第二届会议将邀请国外和国内著名专家做专题讲座，针对国际国内肝胆胰外科进展及近年来的热点、难点问题进行讨论；并交流诊治经验，推广新理论、新技术、新方法，了解国内外肝胆胰疾病诊断、治疗发展趋势；同时放映手术录像. 大会热烈欢迎全国各地肝胆胰领域的内科、外科、影像科各级医师以及科研人员积极投稿和报名参加.

会议同时召开第三届全国普外科主任论坛，因此也欢迎从事医疗卫生管理的各级医院正、副院长及正、副主任积极投稿和报名参加.

本次会议已列入 2006 年国家级继续医学教育项目，参会代表均授予国家级继续医学教育学分 10 分.

来稿要求：寄全文及 500-800 字论文摘要，同时寄论文的软盘一份或发电子邮件. 以附件的形式发送至 chenxp@medmail.com.cn，也可将稿件打印后寄至：武汉市解放大道 1095 号，武汉华中科技大学附属同济医院肝胆胰外科研究所张志伟、黄志勇副教授（收），邮编：430030；联系电话：027-83662599.（世界胃肠病学杂志社 2005-09-15）