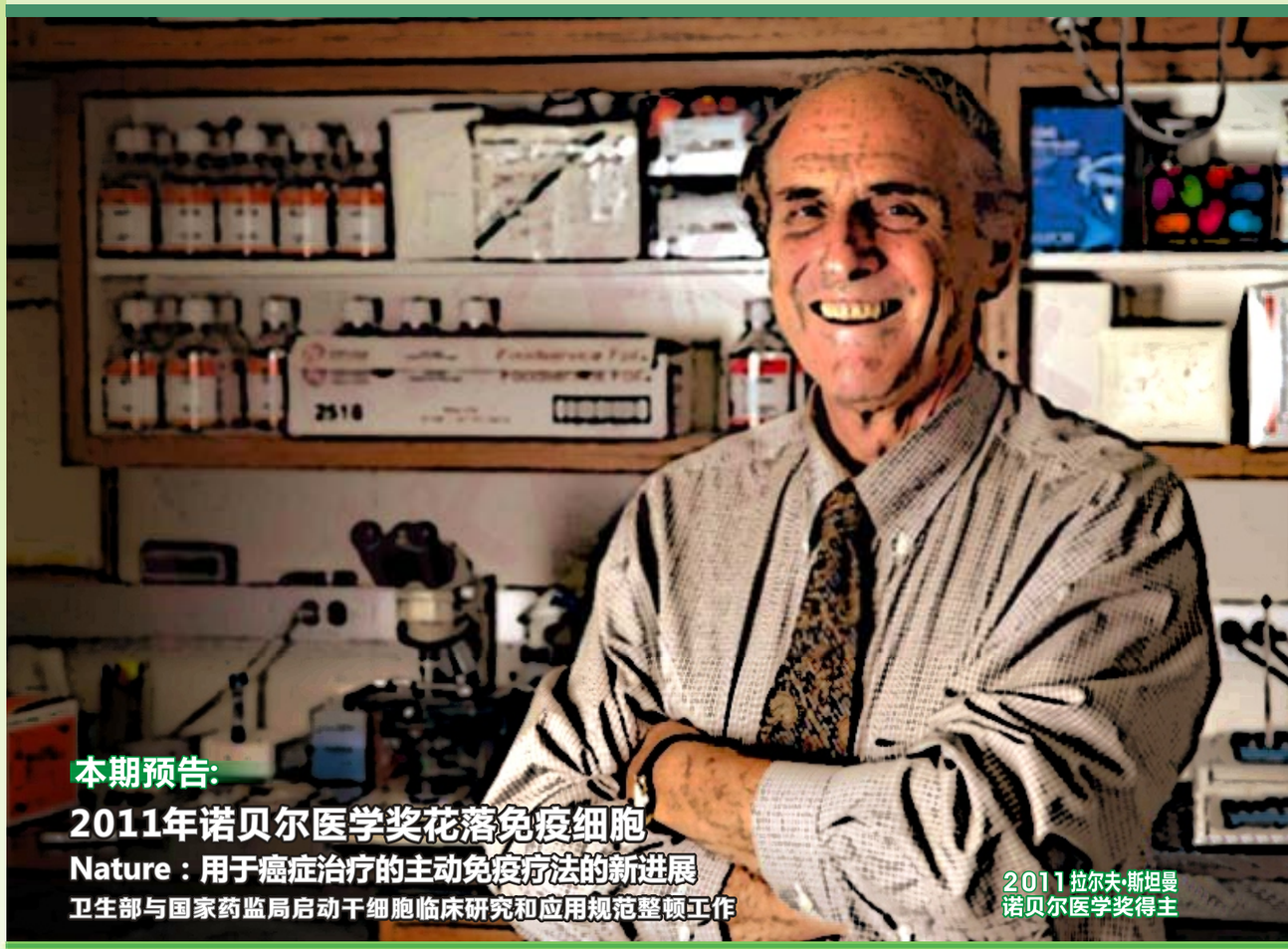
HORNETCORN
BIOTECHNOLOGY

中国生物治疗网：www.chinaswzl.com
电话　：0755-21672023-617
传真　：0755-21672020
Email：info@hornetcorn.com
地址　：深圳市高新技术产业园清华紫光信息港B座406

合一康生物 学术内刊
ACADEMIC JOURNAL



本期预告:

2011年诺贝尔医学奖花落免疫细胞

Nature：用于癌症治疗的主动免疫疗法的新进展

卫生部与国家药监局启动干细胞临床研究和应用规范整顿工作

2011 拉尔夫·斯坦曼
诺贝尔医学奖得主

本期推荐文献

* Transfusion of autologous cytokine-induced killer cells inhibits viral replication in patients with chronic hepatitis B virus infection
* A randomized, controlled trial of postoperative adjuvant cytokine-induced killer cells immunotherapy after radicalresection of hepatocellular carcinoma

总02期
（内部资料　仅供交流）

深　圳　市　合　一　康　生　物　科　技　有　限　公　司

行业新闻

2011年诺贝尔医学奖花落免疫细胞

来源：诺贝尔奖网站
2011-10-03

北京时间10月3日下午5点30分，2011年诺贝尔生理学或医学奖揭晓，美国、法国三位科学家因在免疫学方面的发现获奖。其中一半的奖金归于Bruce A. Beutler和Jules A. Hoffmann，获奖理由是“先天免疫激活方面的发现”；另一半奖金归于Ralph M. Steinman，获奖理由是“发现树枝状细胞及其在获得性免疫中的作用”。

今年的诺奖得主发现了免疫系统激活的关键原理，从而彻底革新了我们对免疫系统的认识。

免疫应答作为一种能帮助人类与其它动物抵御细菌及其它微生物的生理过程，长久以来，科学家们一直在寻找它的“守护者”。Bruce Beutler和Jules Hoffmann发现了能识别微生物并激活先天性免疫的受体蛋白质，从而揭示了身体免疫应答过程的第一步。Ralph Steinman则发现了免疫系统中的树突状细胞，以及其可激活并控制获得性免疫的功能，从而完成身体免疫应答过程的下一步，即将微生物清除出体内。

三位诺奖得主的发现揭示了免疫应答中的先天性免疫和获得性免疫是如何被激活，从而让我们对疾病机理有了一个新的见解。他们的工作为传染病、癌症和炎症的防治开辟了新的道路。

PNAS：研究发现免疫系统中的“维和部队”

来源：科技日报
2011-10-20

美国物理学家组织网10月17日报道，生活在人类皮肤和肠道的细菌比我们自身的细胞还要多，它们大多是人体需要的益生菌。但免疫系统是怎样识别这些“非自体”细菌而不伤害它们呢？澳大利亚悉尼大学世纪学院的科学家发现，在皮肤外层的免疫细胞中有一群“维和部队”，它们阻止了免疫系统攻击有益细菌。该研究有望为诸如炎性肠道疾病等免疫调节类疾病带来新的疗法。论文发表在近日的美国《国家科学院院刊》（PNAS）上。

研究人员在论文中指出，表层皮肤中的免疫细胞其通常职责是作为维和部队，阻止免疫系统的正常反应。实验中，当研究人员想刺激表皮产生免疫反应时，一种叫做郎格汉斯细胞（Langerhans cells）的树突状细胞，抑制了每一次免疫可能。

“我们想激活较长时间的免疫反应，但无论我们引入什么，郎格汉斯细胞总是能诱导出免疫容忍。”芭芭拉·法泽卡斯·圣格罗斯教授说，这一结果好像和主流的免疫理论正相反。主流免疫理论认为，树突状细胞会吞噬细菌、病毒或其他外来入侵者，并给这些外来物贴上抗原标签，抗原能与其他免疫细胞结合，会改变通过的T细胞而引发瀑布式的反应，最终使任何带有抗原标记的物质都被消灭。

然而，研究小组发现，郎格汉斯细胞和其他的树突状细胞有很大不同：它们在激活辅助性T细胞（helper T cells）后，会告诉它们自我毁灭。法泽卡斯解释说，这和人们通常认为的相反。以前人们认为，如果出现了一次活性反应，就是开始了长期免疫反应；而事实上，免疫系统的防御是分层次的，表皮下面的一层中有各种不同类型的树突状细胞，它们被安排做随后的抗菌反应。所以，只有细菌穿越表皮到达更深处遇上这些细胞，才会引发免疫反应杀死它们。

澳大利亚的炎性肠道疾病发病率是世界最高的。研究人员指出，在这类疾病中，免疫系统被激活来抵抗这些肠道细菌。他们的发现也有助于找出发生这种紊乱的原因，并找到治疗各种免疫系统疾病的方法。

法泽卡斯说：“我们仅仅是模仿免疫系统的某些功能，比如接种疫苗，就能发挥很大作用。如果能模拟郎格汉斯细胞的功能，就能开发出像皮肤免疫系统那样高级的疗法，精确容忍某种特殊的抗原。”

Nature：清除老化细胞或能延缓衰老

美国研究人员通过清除老化细胞让小鼠青春常驻，推迟显现皱纹、肌肉萎缩、白内障等老化征兆。
研究人员认为，这一结果有望用于老年人护理。专家称，这只是初步研究，结果虽然“令人着迷”，但须审慎看待。

缓衰老

研究主要针对老化细胞。这种细胞不再分裂出新细胞，在防止肿瘤生长方面起着重要作用。老化细胞由免疫系统清理，随着时间推移，在身体积聚数量增多。研究人员估计，高龄老人全身大约10%的细胞为老化细胞。梅奥诊所研究人员开发一种药物，能够杀死小鼠身上所有老化细胞。研究人员首先通过基因工程培育特殊小鼠，使它们的成熟老化速度快于正常同类。

研究人员观察三种老化征兆，即眼内白内障形成、肌肉组织萎缩和皮下脂肪堆积。结果发现，如果小鼠在老化迹象出现之前摄取药物，三种征兆“神奇地延迟”；如果小鼠在老化迹象开始后摄取药物，肌肉功能得到改善。

不增寿

英国广播公司（BBC）11月2日援引研究人员之一詹姆斯•柯克兰的话报道：“我从未见过这样的事。”

另一研究人员扬•范德乌森说：“我们对效果深感惊讶，它意义深远。”他对这种药物的影响感到乐观，认为这种药有助老年人健康地度过晚年生活。在他看来，“如果生活质量差，没有人愿意长寿”。这种药物对小鼠没有延年益寿的效果。研究人员认为，这可能因为小鼠经过基因改造。

研究结果由英国《自然》杂志发表。

来源：新华网
2011-11-04

须审慎

这项研究结果向人们展示了延缓衰老的美好前景，不过老化细胞无法完全清除。范德乌森说，年轻人能够清除老化细胞，“你须审慎看待可以强化免疫系统，确保老化细胞得到清理”；或者可以发明一种药，专门针对老化细胞，因为这种细胞产生一种独一无二的蛋白质。英国医学研究委员会临床学中心的吉泽斯•吉尔博士说，梅奥诊所研究人员的认定需要“审慎对待，这只是一项初步研究”。他说，不过这是一项“迷人”的研究，显示“如果能够清除老化细胞，就可以改善老化带来的（身体特征）表现型，提高老龄人群生活质量”。

美科学家发现
受者免疫系统控制着干细胞再生

nauralkillerce导语：发表在Nature Medicine期刊上一篇新研究论文描述了不同类型的免疫系统T细胞交替地阻止和鼓励干细胞再生骨和组织，从而将干细胞移植受者的免疫系统在基于干细胞再生上的重要性变成引人注目的焦点。现编译如下；

Nature Medicine
2011-11-27

研究成果：

在美国南加州大学Ostrow牙科学院颅面分子生物学中心开展的一项研究调查了含有骨缺陷的小鼠如何对骨髓间充质干细胞(bone marrow mesenchymal stem cell, BMMSC)介导的骨再生做出反应。

正常情况下，小鼠的T细胞产生一种炎性反应，引发细胞因子干扰素γ (INF-γ)和肿瘤坏死因子α (TNF-α)产生。它们攻击和杀死这些干细胞，从而阻止新骨产生。这就意味着尽管T细胞能够保护身体免受感染，但同时也能够阻止组织健康再生。

然而，当对这些小鼠灌注调节新T细胞(regulatory T-cells, Tregs)后，这些干扰性的INF-γ和TNF-α水平下降，从而增加骨再生和修复骨缺陷的比率。再者，在骨缺陷位点施加抗炎性药物阿司匹林也可增加BMMSC能够再生骨的比率。

研究意义：

博士后研究助理和第一作者Yi Liu认为这些发现清楚说明了T细胞在组织再生中发挥着以前没有认识到的作用，这也意味着科学家们在探索基于干细胞再生的可能性时需要将他们的关注重心调整到免疫系统上。

原文信息：Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-γ and TNF-α.
Yi Liu ,et al.Nature Medicine, 2011 DOI: 10.1038/nm.254
Http://www.nature.com/nm/journal/vaop/ncurrent/full/nm.2542.html

Nature：癌变前的衰老监测可限制肝癌发展

Nature asia
2011-11-24

11月24日，nature杂志在线发表了德国研究人员的最新研究成果，揭示了肝细胞癌变前的衰老监测可以限制肝癌的发展。致癌基因诱导的衰老曾被发现起一个内在肿瘤抑制机制的作用。Lars Zender及其同事提出了“衰老监测”的概念：他们发现，恶变前的衰老肝细胞能通过一个由肿瘤抗原引导的免疫反应被清除。这个过程需要CD4+ T细胞的参与，并且在小鼠模型中抑制肝癌的发展。本文作者们还提供了这样的证据：衰老肝细胞会在免疫系统被抑制的患者肝脏中积累，说明衰老监测对人类也可能行得通。设计用来利用对恶变前的衰老细胞进行抗原特异性免疫监测的策略在癌症预防和治疗中可能会有潜力，而这种类型的抗原特异性免疫反应在疫苗生产中也可能会有用。

Senescence surveillance of pre-malignant hepatocytes limits liver cancer development

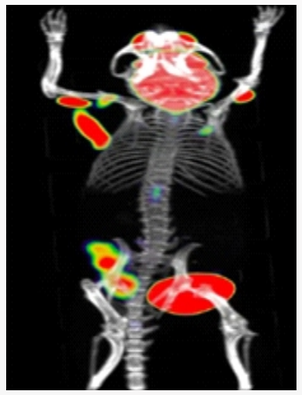
Upon the aberrant activation of oncogenes, normal cells can enter the cellular senescence program, a state of stable cell-cycle arrest, which represents an important barrier against tumour development in vivo. Senescent cells communicate with their environment by secreting various cytokines and growth factors, and it was reported that this 'secretory phenotype' can have pro- as well as anti-tumorigenic effects. Here we show that oncogene-induced senescence occurs in otherwise normal murine hepatocytes in vivo. Pre-malignant senescent hepatocytes secrete chemo- and cytokines and are subject to immune-mediated clearance (designated as 'senescence surveillance'), which depends on an intact CD4+ T-cell-mediated adaptive immune response. Impaired immune surveillance of pre-malignant senescent hepatocytes results in the development of murine hepatocellular carcinomas (HCCs), thus showing that senescence surveillance is important for tumour suppression in vivo. In accordance with these observations, ras-specific Th1 lymphocytes could be detected in mice, in which oncogene-induced senescence had been triggered by hepatic expression of NrasG12V. We also found that CD4+ T cells require monocytes/macrophages to execute the clearance of senescent hepatocytes. Our study indicates that senescence surveillance represents an important extrinsic component of the senescence anti-tumour barrier, and illustrates how the cellular senescence program is involved in tumour immune surveillance by mounting specific immune responses against antigens expressed in pre-malignant senescent cells.

PNAS：小鼠模型可产生肿瘤特异性人类T细胞

来源：生物谷
2011-12-06

近日一篇发表在美国国家科学院院刊（Proceedings of the National Academy of Sciences）的文章称，研究者通过基因工程技术研制出了可产生特异性抗肿瘤人类T淋巴细胞的小鼠模型，该模型或可成为人类肿瘤研究的重要方法。

有研究证明经基因修饰的T细胞受体（TCR）可识别并攻击特定的肿瘤细胞，但其过程较为复杂且无法产生稳定并具有自我更新能力的T细胞。因此本文的研究者制造出了可在其体内产生特异性抗黑素瘤的人类T淋巴细胞的小鼠模型，该T细胞由经过基因修饰的人类造血干细胞发展而来。



此前有人制造了可产生HIV特异性T细胞模型，但由于该模型无法完全重建人类细胞而导致其结果无法在体内进行检测。研究者于是对该模型进行了改进，他们通过将一个有功能的人类胸腺和经黑素瘤特异性TCR转导的人类造血祖细胞结合在一起修复了该缺陷。

随后研究者以黑素瘤为检测对象进行检测，结果发现大多数小鼠体内的肿瘤都得到了清除，或者体积缩小。而且，这些转导的人类细胞在移植4个月后仍可以检测到，表明该干细胞的重建一旦启动便可长期存在。

该研究表明，将人类造血祖细胞使用基因工程的方法进行修饰，或许可以成为人类对抗慢性疾病如肿瘤的新武器。

修改血液干细胞基因可清除黑色素瘤

来源：新华网
2011-12-05

据美国物理学家组织网报道，美国科学家首次通过实验证明，可以在活的生物体体内对血液干细胞进行基因修改，让其变成“癌症杀手”T细胞，使用该方法治疗人类黑色素瘤的治愈率高达40%。研究发表在近日出版的《美国国家科学院院刊》上。

加州大学洛杉矶分校癌症和干细胞研究中心的科学家在该校医学、微生物学、分子遗传学教授杰罗姆•扎克的领导下，从一位黑色素瘤病患体内提取出T细胞受体（其能找出被黑色素瘤表达的抗原）的基因，并用一个病毒载体将该基因引入人体血液干细胞的细胞核中，对血液干细胞进行了遗传修改。随后，他们将经过遗传修改的血液干细胞置于人的胸腺组织中，并将其移入老鼠体内，以便研究在一个活的生物体内人的免疫系统对黑色素瘤的反应。结果发现，大约6周后，经过遗传修改的血液干细胞发育为大量成熟的能有效对付黑色素瘤的T细胞。

该研究的第一作者、扎克实验室的研究员迪米特里奥斯•凡塔克斯表示，这些基因会同细胞的DNA（脱氧核糖核酸）合为一体并永久并入血液干细胞中，从理论上来说，这意味着这些血液干细胞能在需要时产生无限多的T细胞来对抗黑色素瘤。而且，少许干细胞就能变为一个T细胞“军队”，在黑色素瘤出现时作出反击。

科学家们将两种黑色素瘤移入9只实验老鼠体内：一种能表达可吸引经过遗传修改的T细胞的抗原；另一种则不能表达该抗原。随后，他们观察了肿瘤大小的变化，并使用正电子放射断层造影术（PET）监测了癌症的代谢活动，结果发现，有4只老鼠体内表达抗原的黑色素瘤被完全清除，其它5只老鼠体内表达抗原的黑色素瘤也变小了。

扎克表示，科学家们可以对T细胞进行遗传修改来对抗疾病，但在多数情况下，T细胞的功效并不持久，需要源源不断地提供更多经过遗传修改的T细胞。而最新方法则通过对血液干细胞进行遗传修改来产生T细胞，可以在需要的时候产生大量“新鲜”的抗癌细胞，也有望确保癌症不复发，因此，这种对免疫系统进行遗传修改的方法意义重大。

该研究团队接下来准备进行临床试验以测试这种方法的效果。一个可能的方法是对周边T细胞和能够产生T细胞的血液干细胞同时进行遗传修改，让周边T细胞作为抗癌的“先锋官”，血液干细胞则作为第二波“勇士”，在前线T细胞慢慢失效时，接手进行战斗。扎克也希望这种对免疫系统进行遗传的方法可用于治疗乳腺癌等其他癌症。

科技部“十二五”生物技术发展规划发布
免疫细胞治疗或将成解决肿瘤突破点

来源：科技部
2011-12-02

科技部于近日发布了《“十二五”生物技术发展规划》（以下简称《规划》）。《规划》指出生物技术是当今国际科技发展的主要推动力，是当今世界高技术发展最快的领域之一，已成为国际竞争的焦点，将成为解决人类社会发展面临的健康、粮食、能源、环境、生物安全等重大问题的突破点。

《规划》的发展目标是“十二五”期间，生物技术自主创新能力显著提升，生物技术整体水平进入世界先进行列，部分领域达到世界领先水平。生物医药、生物农业、生物制造、生物能源、生物环保等产业快速崛起，生物产业整体布局基本形成，推动生物产业成为国民经济支柱产业之一，使我国成为生物技术强国和生物产业大国。

《规划》指出，紧跟国际生物技术发展前沿，瞄准国家重大战略需求，根据国家各大科技计划的侧重点，兼顾现实和中长期发展，整合覆盖医药、农业、制造、能源、环保等各领域的资源，原始创新与集成创新相结合，引进、吸收、消化、再创新相结合，加强协同创新，形成自主核心技术，培育原始创新成果，形成可持续发展能力。集成各部门和地方的力量，整体规划，分步实施，以点带面，体现基础研究、应用研究和产业化的衔接。

《规划》还提出，面对我国经济社会发展方式的转变和新一轮科技革命带来的挑战，通过一系列的保障措施，打造国际一流水平的国家重点实验室、国家工程技术研究中心、研究共享平台和产业化示范基地，加强农业科学、人口健康科学、工业生物科学各领域前瞻性基础研究；突破“组学”技术、合成生物学技术、生物信息技术、干细胞与再生医学技术、基因治疗与细胞治疗技术、分子分型与个体化诊疗技术、生物芯片与生物影像技术、生物过程工程技术、生物催化工程技术、药靶发现与药物分子设计技术、动植物品种设计技术、生物安全关键技术等核心关键技术；重点研究开发生物医药、生物农业、生物制造、生物能源和生物环保的重大产品和技术系统。

PNAS：干细胞衰老可致白血病患病率升高

近日，国际著名杂志《国家科学院院刊》PNAS刊登了斯坦福大学研究人员的最新研究成果“Human bone marrow hematopoieticstem cells are increased in frequency and myeloid-biased with age”，参与了斯坦福大学医学科学的培训计划的Pang，是本研究的第一作者；病理学教授Irving Weissman博士为通讯作者，Weissman还是斯坦福大学干细胞生物学和再生医学研究所主任。

据美国斯坦福大学医学院的科学家称，人类干细胞也不能幸免于细胞衰老。研究人员对可生成血液和免疫系统细胞的造血干细胞进行了研究。了解随着时间的流逝这些干细胞何时以及如何开始衰老可能解释为什么一些疾病（如急性髓细胞性白血病）患病率随着年龄的增加而升高，以及为什么老年人往往更容易感染，如感冒和流感。

Wendy Pang博士说，“我们知道，免疫系统功能似乎随着年龄的增加而下降。”“这是对年轻人和老年人提纯的造血干细胞功能和基因表达谱进行比较的首次研究，该研究告诉我们，这些临床变化可以追溯到干细胞功能。”

具体来说，研究人员发现，与20岁-35岁的健康人相比，65岁以上的健康人的造血干细胞生成淋巴细胞较少，而淋巴细胞可调节对病毒和细菌的免疫应答逐渐增加（细胞分离自骨髓标本）。相反，老年人的造血干细胞，更易于生成另一种称之为骨髓细胞的白细胞。这种倾向也许可以解释为什么老年人比年轻人更易生成骨髓恶性肿瘤。

Pang开始进行研究以了解人造血干细胞的老化是否像小鼠造血干细胞一样。以往的研究表明，随着实验室老鼠衰老，小鼠造血干细胞在数量和功能发生改变。Pang采集了15名健康老人和28位健康年轻人的造血干细胞并对他们的患病率，分布及细胞周期谱进行了比较。

她发现，老年人的造血干细胞中的骨髓细胞比例比年轻人高。老年人的造血干细胞分裂比年轻人更活跃。但其数量更多，且增殖速度加快并不能转化为更大的效益；就如同顶部旋转减慢后顶部出现摇摆而失去控制一样，老化的造血干细胞在尝试保持与日常生活的需求上出现失控。

当Pang在实验室培养皿中纯化并增殖造血干细胞时发现，老年人的造血干细胞分化成B淋巴细胞较少，而更易分化成髓细胞。此外，免疫缺陷的实验室小鼠移植老年人造血干细胞后数周到数月，其骨髓中的人造血干细胞分化为较大比例的骨髓至淋巴样细胞。

最后，Pang对两组人造血干细胞基因表达谱，以及5份42-61岁人类造血干细胞样本进行了研究。她发现，老人捐助者的造血干细胞表达几种与细胞周期，增殖和发展有关的年龄相关基因，以及与DNA修复和细胞死亡相关的基因。上述基因表达水平较高，表明这些细胞在新的血液或免疫细胞需要，而非适时的进入细胞周期前不太可能寂然无声。

总体来说，结果反映了不同年龄的实验室小鼠造血干细胞的研究结果。他们认为，人造血干细胞的功能随着一个人的年龄变化而改变，就是说有时会不但会导致免疫应答不足，而且还会引起不适当的增殖和特定类型的血液癌症如急性髓细胞性白血病。他们还为许多其他条件下的研究贡献了有价值的信息。

弗吉尼亚和D.K. Ludwig临床癌症研究中心的教授，斯坦福大学癌症研究所的成员Weissman说，“在小鼠和人类中，体系老化的机制一直是难题”。“因为年龄较大的小鼠和人的造血干细胞来自于早期的造血干细胞，有两种可能可解释这些差异是如何发生的，一是随着衰老，早期的造血干细胞的基因表达模式发生改变，出现遗传性改变，从而向髓系发展，二是每个早期造血干细胞已经有了一个特异性谱系，并通过年龄自然选择争取宝贵的微环境，而向髓细胞方向发展。”Weissman认为，理解这种可能正确的理论能够帮助未来的医生保证与年龄相关疾病患者的更合适的造血干细胞存活。

Pang说，“这些调查结果也将作为未来与年龄有关的疾病研究如髓细胞发育不良综合征，贫血和白血病的重要的基线数据，现在我们知道了未生病的老年个体的造血干细胞如何变化以及其功能，我们应该能够从正常年龄有关的疾病中梳理出与疾病相关的变化。”

来源：医脉通
2011-12-06

Nature：用于癌症治疗的主动免疫疗法的新进展

Nature asia
2011-12-22

12月22日，国际著名杂志Nature刊登了国外研究人员的最新研究成果“Cancer immunotherapy comes of age。”文章中，研究者揭示了用于癌症治疗的主动免疫疗法的最新进展。

利用单克隆抗体和供体T细胞进行的被动免疫疗法对某些类型的癌症是有效的，但尽管人们进行了广泛研究，对特定的、有耐久性的抗肿瘤免疫实施主动刺激仍然难以实现。最近，随着用于前列腺癌的sipuleucel-T疗法和用于一些转移性黑素瘤的ipilimumab药物的研发成功，这种情况已经得到改变。这些成果使人们对癌症免疫疗法重新产生了兴趣。这篇Review文章总结了有关疫苗、T细胞免疫调控因子和其他活性免疫刺激药物的最新研究工作，这些药物与定向疗法相结合，可能会导致未来癌症治疗方法的问世。

Cancer immunotherapy comes of age

Ira Mellman,George Coukos& Glenn Dranoff

Activating the immune system for therapeutic benefit in cancer has long been a goal in immunology and oncology. After decades of disappointment, the tide has finally changed due to the success of recent proof-of-concept clinical trials. Most notable has been the ability of the anti-CTLA4 antibody, ipilimumab, to achieve a significant increase in survival for patients with metastatic melanoma, for which conventional therapies have failed. In the context of advances in the understanding of how tolerance, immunity and immunosuppression regulate antitumour immune responses together with the advent of targeted therapies, these successes suggest that active immunotherapy represents a path to obtain a durable and long-lasting response in cancer patients.

卫生部与国家药监局启动干细胞临床研究和应用规范整顿工作

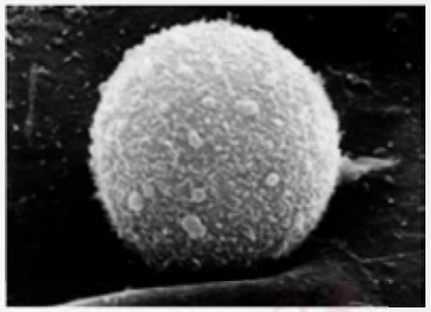
中华人民共和国卫生部
2012-01-06

为促进干细胞治疗技术科学、有序地发展，规范干细胞临床研究和应用行为，整顿干细胞治疗工作。日前，卫生部与国家食品药品监督管理局联合下发《关于开展干细胞临床研究和应用自查自纠工作的通知》，决定开展为期一年的干细胞临床研究和应用规范整顿工作。为加强此项工作的组织领导，卫生部和国家药监局已经成立了干细胞临床研究和应用规范整顿工作领导小组，该工作将分为自查自纠、重新认证和规范管理等阶段。

自查自纠阶段要求全国各级各类从事干细胞临床研究和应用的医疗机构及相关研制单位，应当按照《药物临床试验质量管理规范》和《医疗技术临床应用管理办法》，开展干细胞临床研究和应用项目（暂不包括未经体外处理的骨髓移植）自查自纠工作。各省级卫生行政部门要对正在开展的干细胞临床研究和应用项目进行认真清理。停止未经卫生部和国家药监局批准的干细胞临床研究和应用活动。对于已经国家药监局批准的干细胞制品临床试验项目，应当严格按照临床试验批件以及《药品临床试验质量管理规范》的要求进行，不得随意变更临床试验方案，更不得自行转变为医疗机构收费项目。2012年7月1日前，暂不受理任何申报项目。在此期间，有关部门将依据现行法律规范，抓紧研究并提出制度性文件草案和相关技术标准、规范，并结合自查自纠工作实际，探索建立适合我国的干细胞临床研究和应用监管模式和长效机制。

卫生部链接：http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohkjjys/s3582/201201/53890.htm

Science Signaling：自我调节抑制机体对癌症的防御作用



据12月21日，每日科学报道，调节性T细胞（Treg细胞），是人体免疫系统组成的一部分，下调其他免疫细胞的活性以防止出现自身免疫性疾病或过敏反应。德国癌症研究中心（DKFZ）的科学家目前已发现在免疫细胞中被Treg细胞阻断的激活步骤。鉴于Treg细胞也能抑制机体对癌症的免疫防御，DKFZ的研究人员获得的这些发现对于开发更有效的癌症疗法是非常重要的。

人体自身的免疫系统不会过激反应，这是至关重要的。如果其关键成员，辅助性T细胞失控了，将导致自身免疫性疾病或过敏。免疫系统如果对传染性病原体反应过激，甚至可能直接损害器官和组织。

称为调节性T细胞（Treg细胞）的一种免疫细胞，确保了免疫反应以协调的方式发生。它们下调辅助T细胞的分裂活动并减少它们产生的免疫介质。“这是通过Treg细胞和辅助T细胞的直接接触达到的”，DKFZ的Peter Krammer教授说，“但是到目前为止我们仍不清楚这种接触作用究竟在辅助性细胞中引发了什么。”研究人员的假设是，与Treg细胞的接触影响了复杂信号级联反应中某些特定的步骤，结果导致了辅助性T细胞的激活。

如果T细胞受体，一种位于辅助性T细胞表面的传感器分子，感应到外援蛋白或受损的蛋白分子，这将触发一个生化激活反应的级联反应。在信号级联的终末，免疫攻击所需的基因将在辅助性细胞的细胞核内开始转录。

联合数个德国科研机构的研究人员，Peter Krammer、Angelika Schmidt及其同事们目前已比较了接触或未接触Treg细胞时辅助性细胞内的信号级联反应。免疫学家发现，在培养皿中两种细胞短暂的接触就足以抑制辅助性T细胞。Treg细胞接触后，典型的释放钙离子进入辅助性细胞胞浆这一过程并没有发生。结果，两个很重要的转录因子，NFkappa8和NFAT，不再起作用。它们通常会激活免疫介质的基因，以提醒免疫系统。

“Treg细胞的反应模式对于癌症药物具有重要的意义。我们很多同事都发现，在各种癌细胞中，Treg细胞能够下调对肿瘤的免疫反应，使转化细胞能够逃脱免疫防御，这为癌症的形成和扩散做出了贡献。因此，我们正寻找一些方法，重新激活这些受抑制的辅助性细胞”，Krammer说，解释了他的工作目标。为了开发对抗癌症的免疫疗法，了解Treg细胞是如何工作同样至关重要。研究人员目前正试图阻止Treg细胞对培养皿中已激活的抗癌免疫细胞的即刻再次抑制作用。

来源：生物谷
2011-12-27

会议通知与回顾

首届国际肿瘤免疫治疗论坛启动 亚洲细胞治疗学会建中国分会

2011年10月21日-23日，第一届国际肿瘤免疫治疗（北京）论坛暨亚洲细胞治疗学会中国分会筹备会在北京召开，这次高水平论坛的组织者包括美国国家癌症顾问委员会委员、美国杜克大学医学院的Kim Lyerly教授，中国工程院副院长、第四军医大学校长樊代明院士，日本东京大学医学研究所所长、亚洲细胞治疗学会会长下坂皓洋，北京大学肿瘤医院内科教授，北京市卫生系统高层次肿瘤内科学科带头人任军教授。与会的100多位来自全国的高水平肿瘤专家就该项技术进行了广泛交流，并将筹备成立亚洲细胞治疗协会中国分会。该组织成立后将在国内开展一系列高水平的国际临床协作项目，预示着国内肿瘤细胞免疫治疗水平将进入跨越式发展阶段。

肿瘤细胞免疫治疗，又称肿瘤生物治疗方法，即将经处理的自体或异体的免疫细胞或免疫分子输给患者，恢复与增强肿瘤患者自身的免疫监测和杀瘤功能，有效地杀灭患者术后和放化疗后体内残存的肿瘤细胞，达到治疗肿瘤、预防复发与转移和最终根治肿瘤的目的，具有特异性强、副作用轻等优点。

自1985年，美国国立卫生研究院（NIH）就把肿瘤的生物治疗列入肿瘤综合治疗的第四大模式。2000年美国举行的“国际肿瘤生物治疗机基因治疗年会”也指出：生物治疗是目前知道的唯一有望完全消灭癌细胞的治疗手段，21世纪是肿瘤生物治疗的世纪。美国杜克大学在细胞免疫治疗领域的研究成果是美国在这类技术上领先优势的代表，Kim Lyerly教授在会上强调，“肿瘤细胞免疫治疗手段在不断的成 熟和完善，但是要得到很好的运用则需要巨大的临床科研投入，建立肿瘤免疫治疗的标准化模式以及制定一系列相关法规。杜克大学愿意对中国在该领域的进步发挥越来越积极的作用。”

中国十二五计划将生物医药产业列为国家重点发展和扶持的新兴战略产业，肿瘤免疫治疗技术在中国无疑面临一个良好的发展机遇，国内外专家均认为中国未来有机会在该领域达到甚至领先于世界水平。任军教授表示：“经过国内同行专家的不懈努力，中国在肿瘤细胞免疫治疗的基础研究上取得了很多进展，但就我国目前的临床治疗水平而言仍然落后国外至少5到10年，具体表现在：治疗的开展是小规模的，主要依托于各个医院的实验室进行细胞制备和处理；细胞制备和处理缺乏标准化操作程序；细胞因子和细胞培养基等细胞制备产品相对缺乏；基础研究和临床治疗脱节。”

据了解，虽然国内肿瘤细胞免疫治疗的技术在进步，市场容量迅速扩大，但由于国内的细胞制备处理散落分布于各个医院，导致各自采用的技术方法、操作流程、质控管理无法统一。这些特征将严重阻碍技术的进步，同时也导致难以形成高水平的公信力的乃至国际公认的研究结果。建立高水平临床治疗协作组织并引入国际成熟技术方法和质量控制体系、全面提升国内的治疗水平迫在眉睫。”

作为本次论坛另一主旨，亚洲细胞治疗学会（ACTO）中国分会的正进入筹备阶段，并有望在年底正式成立。这将是中国地区第一个具有国际水平的肿瘤细胞免疫治疗临床协作专业组织，它将帮助中国迈出肿瘤细胞免疫治疗标准化的第一步。

亚洲细胞治疗学会会长下坂皓洋在会上分享了ACTO中国分会在未来1~2年内的发展构想：“ACTO中国分会将致力于中国地区肿瘤细胞免疫治疗技术的进步，推动标准化操作程序和质量控制体系的形成；同时ACTO中国分会将协调、组织国际一流的学术组织，如杜克大学肿瘤中心等单位与中国开展一系列国际临床协作项目。”

北京大学肿瘤医院内科教授，北京市卫生系统高层次肿瘤内科学科带头人任军教授


美国国家癌症顾问委员会委员、美国杜克大学医学院的Kim Lyerly教授

第十二届全国肿瘤生物治疗学术会议圆满落幕

2011年10月8-10日，由＂中国免疫学会肿瘤免疫与生物治疗分会＂和＂中国抗癌协会生物治疗专业委员会＂联合主办,山东大学药学院免疫药理与免疫治疗研究所和《中国肿瘤生物治疗杂志》联合承办的＂第十二届全国肿瘤生物治疗学术会议＂在山东省济南市隆重召开。众多国内外肿瘤专家纷纷前来参加本次会议，并通过知识技术分享、学术报告分析等方式进行零距离的交流。

10日上午8：30分，由于益芝、张彩主持的肿瘤生物治疗学术会议在热烈而轻松的气氛中缓缓拉开帷幕。会议邀请了中国科学技术大学免疫学研究所的田志刚、中科院生物物理研究所感染与免疫中心的王盛典、华中科技大学同济医学院的黄波、中山大学附属肿瘤医院生物治疗中心夏建川教授、山东大学药学院免疫药理与免疫治疗研究所的张建、中国医学科学院肿瘤研究所的张叔人等分别对肿瘤免疫细胞治疗的基础研究及临床运用等做了专题报告。

此外，会议特邀了来自美国西雅图哈金生癌症研究中心(FRED HUTCHINSON CANCER RESEASRCH CENTER)资深科学家李永庆博士做了“癌症介入免疫疗法的人抗体特异性T细胞的制备，分离与扩增”的专题报告。李博士在细胞免疫治疗，尤其是T细胞治疗领域具有世界最前沿技术，其领导的实验室是目前在世界范围内成功治愈多例晚期癌症患者的唯一实验室。该实验室与美国国立研究院(NIH)具有极其密切的学术交流，同时也是美国肿瘤细胞免疫临床试验网(CITN)的最主要研究基地。

在会议上，免疫治疗这个高科技行业得到了专家们的一致认可，肿瘤生物治疗作为一种自身免疫抗癌的新型治疗方法，将是21世界唯一有望治愈肿瘤的、新兴的、且具有显著疗效的肿瘤治疗模式。与会专家的认同，也标志着免疫治疗这个高科技行业将被快速运用到肿瘤的治疗当中，让广大的医务工作者、肿瘤患者在治疗肿瘤方面有了更多的选择和希望。


李永庆博士(MD, PhD)：“癌症介入免疫疗法的人抗体特异性T细胞的制备，分离与扩增”


中国科学技术大学免疫学研究所田志刚：“NK细胞与肿瘤生物治疗”


中国医学科学院肿瘤研究所张叔人:新版《药品生产质量管理规范》和《增强体细胞治疗的医控》

第五届全国肝病免疫和生物治疗研讨会

2012年4月 北京 会议简介

肝脏疾病，一直以来都是诸多科学家、临床工作者期望解决的临床难题。近年来，随着免疫学、病毒学等研究的深入，特别是临床免疫学的进展，对肝脏疾病的研究及治疗起到了巨大的推动。本次会议希望将基础研究与临床治疗紧密结合，着重介绍本领域前沿进展、研究成果和最新技术，主要内容包括：肝病的临床免疫学研究进展，免疫细胞治疗技术，干细胞治疗技术等。旨在通过会议促进专家交流，为基础研究与临床研究有机结合搭建桥梁。

主办单位：中国免疫学分会感染免疫分会、全军传染病学专业委员会。
承办单位：生物谷
Http://www.bioon.com.cn/custom/livermed/20111220/?utm_source=DMdelivery&utm_medium=email&utm_content=DMDlink%201&utm_campaign=livermed-20111220-v1-c002

本期推荐文献

Transfusion of autologous cytokine-induced killer cells inhibits viral replication in patients with chronic hepatitis B virus infection

Ming Shi [a], Junliang Fu [a], Feng Shi [a], Bin Zhang [a], Zirong Tang [a], Lei Jin [a], Zhenping Fan [b], ZhengZhang [a], Liming Chen [b], Huifeng Wang [b], George K.K. Lau [c], Fu-Sheng Wang [a,b,*]

a Research Center for Biological Therapy, Beijing 302 Hospital, Beijing 100039, China
b The 6th Clinical Department, Beijing 302 Hospital, Beijing 100039, China
c Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR, China

自体CIK细胞移植抑制慢乙肝患者病毒复制

文献摘要：

过继免疫治疗在清除慢乙肝（CHB）患者体内乙肝病毒中扮演着重要的作用。但是，CIK细胞是否能抑制CHB患者HBV复制还不清楚，特别是在出现耐药的情况下。本研究运用自体CIK细胞治疗了21例CHB患者，其CIK细胞得到大量增殖，功能增强，在某些患者中，CIK细胞的应用与患者血清HBV病毒载量下降和肝功能改善有密切关系，基线ALT＞40U/L的患者病毒应答率高于基线ALT≤40U/L的患者。此外，患者HBeAg消失或获得血清学转换，其基线ALT水平一般＞40U/L。未观察到严重的副反应。

Introduction

Hepatitis B virus (HBV) infection represents a major public health problem, affecting more than 400 million people worldwide. Substantial evidence exists to indicate that host innate and adaptive immune responses play a crucial role in controlling HBV replication in vivo [1–3]. The currently available therapeutic drugs for patients with chronic hepatitis B virus infection (CHB) include interferon-alpha(IFN-α)-based therapy and nucleoside or nucleotide analogs. Unfortunately, IFN-α-based treatment is effective in only 30% of cases and often has serious side effects [4,5]. Nucleoside analogs, such as lamivudine, adefovir dipivoxil, and entecavir, are capable of inhibiting HBV replication and improving liver histology. However, their efficacy is limited by viral reactivation on discontinuation of treatment. In the case of prolonged drug treatment, formation of viral epitope mutants leads to avoidance of antiviral immunity or development of mutant drug resistance [6–8]. In addition, these nucleoside analogs are virostatic rather than virucidal and, therefore, are incapable of suppressing the cccDNA of the virus within the hepatocytes.

Animal and clinical studies have demonstrated that transfer of HBV-immune memory T cells from an immune donor through bone marrow transplantation (BMT) or transfer of peripheral blood lymphocytes (PBLs) can induce serological clearance of the hepatitis B surface antigen (HBsAg) and lead to seroconversion in patients with CHB [9–11]. However, allogeneic BMT treatment is not a practical therapeutic option for CHB patients, considering its cost and the risks of potentially serious complications[12,13]. Therapeutic vaccines, including an HBV antigen-pulsed DC vaccine and an HBsAg–HBIG complex vaccine, have also been reported to combat immune tolerance and elicit an antiviral CTL response in HBV transgenic mice[14,15]. Some of these vaccines were previously administered in clinical trials for the treatment of hepatitis B [15–17]. However, administration of the HBsAg/AS02 candidate vaccine in combination with lamivudine showed the same efficacy as lamivudine monotherapy in CHB patients [18]. In addition, it was recently reported that transfer of autologous immune cells activated by multiple factors (anti-CD3 monoclonal antibody, interleukin-2 and interferon-γ) inhibited viral replication in CHB patients [19]. Hence, the real efficacy and indications of such an intervention need to be further investigated in a larger sample of individuals with CHB.

Cytokine-induced killer (CIK) cells comprise heterogeneous cell populations including a major effector cell population expressing both the T-cell and NK cell markers (CD3+CD56+). CIK cells can lyse target cells in a non-major histocompatibility (MHC)-restricted manner [20,21]. We previously showed that autologous transfusion of CIK cells can suppress HBV replication in patients with HBV-associated hepatocellular carcinoma (HCC) [22,23]. However, clinical trials have never been conducted with CIK cells in non-cancerous patients, such as individuals with CHB.

The initial aims of this study were to prepare and characterize the CIK cells from CHB patients. Subsequently, we conducted a safety and efficacy trial with autologous CIK cell transfusion in CHB patients, particularly those who had developed drug resistance.

Patients and methods

Patients and study design

A total of 21 CHB patients were enrolled in this clinical trial (Table 1). All patients fulfilled the entry criteria of this trial, and the study was approved by the institutional review board. All patients were >16 years old and had been positive for HBsAg, hepatitis Be antigen (HBeAg), and HBV DNA for more than 6 months. The diagnosis of CHB was further confirmed by liver histology. Of these patients, 6 had previously received antiviral treatment and had developed drug resistance leading to discontinuation of antiviral therapy>16 months before enrollment for CIK cell treatment. The HBV DNA load (median,6.58 log10copies/mL; range,5.08-8.79 log10 copies/mL) and serum ALT levels (median,57 U/L;range, 12-214 U/L) in these patients were maintained at a relatively stable level for at least 6 months before CIK cell treatment. We excluded (1) patients having HCC, autoimmune hepatitis, hepatitis C, hepatitis D,human immunodeficiency virus co-infection, or other serious con-current illnesses; (2) those who had received treatment with immunomodulatory or chronic antiviral therapy within the last 6 months; and (3) pregnant women. All patients were of the HLA genotype HLA-A2+. Written consent was obtained from each participant.

Table 1 Baseline characteristics of CHB patients with CIK cell transfusion.

Patient	Sex (M/F)	Age (Year)	HBV DNA (Copies/mL)	ALT (U/L)	TBIL (μmol/L)	Treatment time	Liver cirrhosis	Immune status*	Drug resistance
1	M	40	2.36×10^{6}	12	47.1	1	+	IT	
2	M	47	2.82×10^{5}	23	29.0	1	–	IT	
3	M	21	3.85×10^{6}	30	8.7	1	–	IT	
4	M	41	3.14×10^{7}	31	5.4	1	–	IT	
5	M	61	2.04×10^{5}	33	12.0	3	–	IT	
6	M	40	6.59×10^{6}	35	10.6	1	–	IT	
7	M	27	3.35×10^{5}	38	18.2	1	–	IT	
8	M	34	2.19×10^{5}	41	3.2	1	+	IA	
9	M	40	2.79×10^{5}	42	14.6	1	+	IA	
10	M	32	6.11×10^{6}	54	9.9	1	–	IA	Lamivudine
11	M	39	1.21×10^{5}	57	12.3	1	+	IA	
12	M	42	2.76×10^{6}	60	26.8	1	–	IA	
13	M	31	3.09×10^{6}	66	13.2	1	–	IA	
14	M	41	1.97×10^{7}	69	7.5	3	–	IA	Lamivudine
15	M	73	1.38×10^{6}	77	16.3	1	+	IA	
16	M	37	1.96×10^{7}	77	6.6	2	–	IA	Lamivudine Adefovir dipivoxil
17	F	40	4.26×10^{7}	80	3.3	2	–	IA	Lamivudine
18	M	32	1.40×10^{7}	92	8.2	1	–	IA	Lamivudine
19	F	35	6.00×10^{8}	140	22.9	1	–	IA	Lamivudine
20	F	27	3.35×10^{6}	210	1.7	1	–	IA	
21	M	35	1.72×10^{8}	214	12.4	1	–	IA	

* IT: Immune tolerance stage, define as $HBsAg^{+}/HBeAg^{+}$, normal serum ALT level, HBV DNA level $\geq 10^{5}$ copies/mL; IA: immune active stage, defined as $HBsAg^{+}/HBeAg^{+}$, abnormal serum ALT level, HBV DNA level $\geq 10^{5}$ copies/mL.

Generation of CIK cell

The CIK cells were generated by the procedure described in our previous report [23]. Briefly, leukapheresis using a blood cell separator (Spectra v 6.1; Cobe, Denver, CO) was conducted in order to obtain peripheral blood mononuclear cells (PBMCs). The PBMCs thus obtained were incubated at a concentration of 2×106/mL in fresh serum-free AIM-V medium (Invitrogen/Gibco, Carlsbad, CA) containing 2000 U/mL of recombinant human gamma interferon (IFN-γ) (Read United Pharmaceutical Co., Beijing, China) in a Lifecell tissue culture flask (Nexell Therapeutics Inc., Arkansas) at 37 °C in a humidified atmosphere of 5% CO2. After incubation for 24 h, anti-CD3 antibody (Center of Immunology Research, Havana, Cuba) and recombinant human interleukin (IL)-2 (Read United Cross Pharmaceutical Co.) were added to the medium at a final concentration of 50 ng/mL and 1000 U/mL, respectively. Fresh AIM-V medium containing IL-2 was replenished after every 2d.CIK cell proliferation, phenotype, and function were analyzed on days 0, 7, 10, 12, and 14 (Fig. 1).

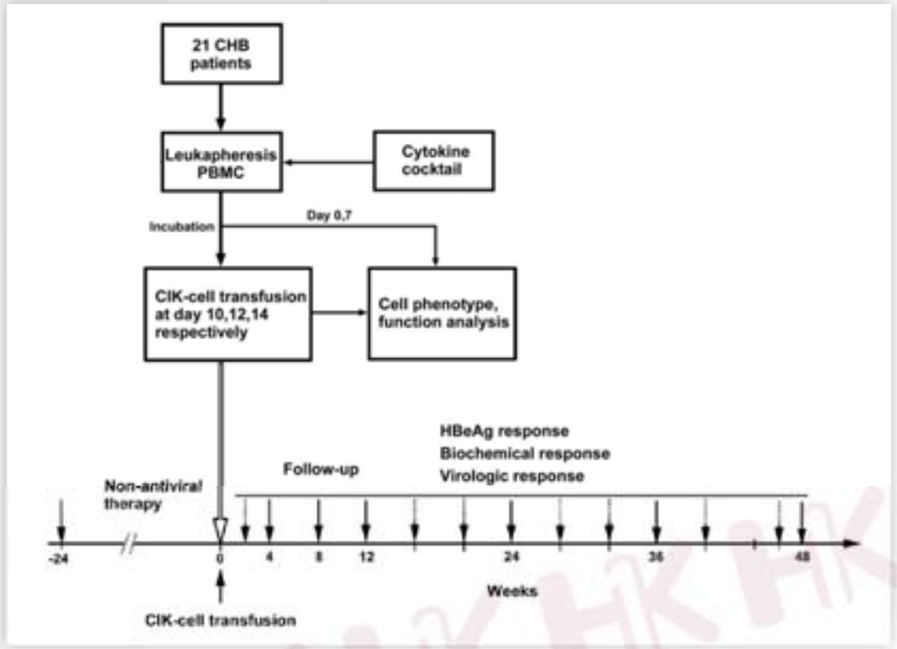


Figure 1
Protocols for the generation and transfusion of autologous CIK cells in each regimen. Using a blood cell separator, a final volume of 50–60 mL of PBMCs was obtained from each CHB patient. CIK cells were induced by incubation of the PBMCs in a cytokine cocktail for 10–14 d. After incubation for 10 d, we began to transfuse the CIK cells back to patients in an autologous manner at 1-d intervals followed by examination over a 48-week follow-up period when HBeAg and biochemical and virological responses were recorded (real line arrow represents all patients, dashed arrow represents some patients in whom HBeAg and biochemical and virological responses were recorded during follow-up).

Analysis of CIK cell subpopulations

In vitro incubated cells were collected and stained with anti-CD3-FITC, and anti-CD4-, anti-CD8-, anti-CD19-,and anti-CD56-PE (eBioscience, San Diego, CA) for 30 min at 4 °C. The cells were then fixed and analyzed using FACSCalibur and CellQuest software (Becton Dickinson, San Jose, CA).

Analysis of programmed death 1 expression on CIK cells

To determine programmed death 1(PD-1) expression, the cells were stained with anti-CD3-PerCP, anti-CD8-PE, anti-CD56-APC, and anti-PD-1-FITC or with isotype-matched control antibodies (eBioscience) for 30 min and were analyzed by flow cytometry as described in our previous report [24].

Analysis of IFN-γ-producing CIK cells

For the analysis of intracellular IFN-γ production, the cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/mL) and monensin (1 μg/mL) for 4 h in the presence of Golgistop agent (10 μg/mL) (eBioscience) followed by washing and staining with antibodies to surface markers (anti-CD3-PerCP, anti-CD8-PE, and anti-CD56-APC). After cells were permeabilized and fixed using Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's instructions, anti-IFN-γ-FITC or isotope-matched control antibodies were added for 30 min followed by flow cytometric analysis.

Analysis of HBV epitope-specific tetramer-positive CD8+ T cells

The HBV-specific CD8+ T cells were analyzed using epitope-specific tetramers. We commercially obtained 3 tetrameric peptide-MHC complexes of the HLA-A0201 allele (Pro Immune, Oxford, UK). In this study, the amino acid sequences of the epitope peptides comprised (1) core amino acids 18–27 FLPSDFFPSI,(2) envelope amino acids 183–191 FLLTRILTI, and (3) polymerase amino acids 575–583 FLLSLGIHL. These epitopes are within the indicated regions of the genotype C of the HBV strain, which is highly prevalent in northern China.

Analysis of degranulation of the CIK cells

CD107a mobilization assay was employed for measuring the degranulation of cells. The cells were stimulated with PMA (50ng/mL), Golgistop agent (10μg/mL), and with anti-CD107a-FITC or isotope-matched control antibodies for 5 h followed by staining with anti-CD3-PerCP, anti-CD8-PE, and anti-CD56-APC for 20 min and flow cytometric analysis.

Study design for administration of CIK cells

The CIK cells were retrieved on days 10, 12, and 14 after commencement of the culture and were administered intravenously to the patients at these 3 time points. Briefly, one half of the incubated CIK cells was harvested by centrifugation and washed twice in physiological saline solution (containing 0.5% human albumin and 100 U/mL of IL-2). Approximately 35×109 cells were resuspended in 400-500mL of the same solution and were intravenously transfused back into the CHB patients. All patients were followed with routine examinations and laboratory tests at outpatient clinic visits for 48 weeks after the last CIK cell transfusion. Virological and biochemical parameters and adverse effects were recorded periodically (at weeks 4, 8,12, 24, 36, and 48 post-treatment, as shown in Fig. 1). A patient was considered to be a virological responder if he was either HBV DNA seronegative or showed 2-log decrease in serum HBV DNA load, and a patient was considered to be a biochemical responder if his serum alanine aminotransferase (ALT) level normalized within 6 months after commencement of CIK cell treatment. Patients who did not fulfill the above requirements were considered to be virological and biochemical non-responders. Serum creatinine and uric acid levels were assayed at baseline and thereafter, during the follow-up period for monitoring renal function. The criterion for multiple transfusions to patients is dependent on the viral load increase for more than one-log compared with that of the last follow-up visit at least 12-weeks prior. No further antiviral or immunoregulation therapy was administered during the follow-up period.

Measurement of serum virolgical markers, HBV DNA levels, and biochemical profiles

Serum HBV DNA was determined by using the ABI Prism 7900HT Sequence Detection System (ABI, Foster City, CA) and validated real-time polymerase chain reaction (PCR) kits with a detection limit of 500 copies/mL (PG Biotech, Shen Zhen City, China) as described in a previous report. Serum HBsAg/anti-HBs and HBeAg/anti-HBe were measured by using ELISA kits (Radim; Pomezia RM, Italy).

Statistical analyses

The data were summarized as the median, mean, standard deviation, and range as applicable and were analyzed using SPSS software. The Wilcoxon matched-pairs t-test was used to compare data from the same individuals. The non-parametric chi-square test was used to compare the virological response rate between 2 groups. Spearman correlation analysis was performed between 2 parameters. For all tests, $P < 0.05$ was considered statistically significant.

Results

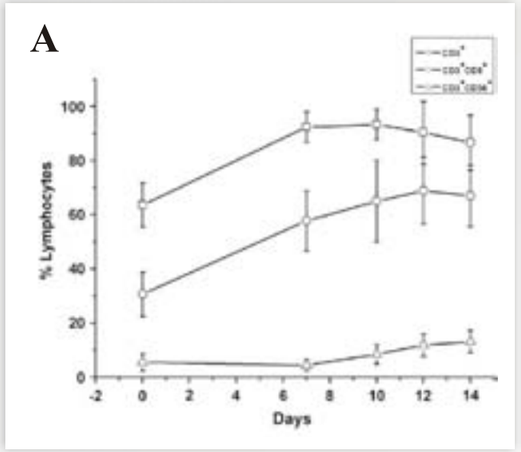
General features of CIK cells in vitro

The CIK cells comprising a heterogeneous population of $CD3^+CD8^+$ and $CD3^+CD56^+$ cells increased significantly after incubation for 14 d (Fig. 2A). During culture with the cytokine cocktail, the number of CIK cells increased(15.2-fold and 16.8-fold for $CD3^+CD8^+$ and $CD3^+CD56^+$ cells, respectively)(Fig. 2B).

The frequencies of IFN-γ-producing $CD3^+CD56^+$ cells significantly increased on days 10, 12, and 14 as compared to those on days 0 and 7. The frequencies of IFN-γ-producing $CD3^+CD8^+$ cells stably increased on days 10, 12,and 14 (Figs.3A–C), and the frequencies of CD107a-expressing $CD3^+CD56^+$ and $CD3^+CD8^+$ cells were also up-regulated on days 10, 12, and 14 (Figs.3D and E). In this culture system, PD-1 expression on $CD3^+CD56^+$ cells peaked on day 7 and subsequently decreased to a lower level on days 12 and 14. In contrast, PD-1 expression on $CD8^+$ T cells obviously decreased and attained a lower level on days 10,12, and 14 (Fig. 3F). Although $CD8^+$ HBV-specific tetramer-positive cells were not detected among the CIK cells (data not shown), our findings indicate that the CIK cells would be suitable for autologous transfusion after in vitro incubation for 10, 12, and 14 d.

Safety of CIK cell transfusion in vivo

Since safety is a major concern regarding adoptive immunotherapy for CHB patients, we monitored serum total protein, albumin, uric acid, creatinine, lactate dehydrogenase, and alkaline phosphatase levels before and after CIK cell transfusion and found that all parameters were within their respective normal ranges. Approximately 85.7% of patients developed a self-limiting fever (body temperature, 37-40°C) within 2-6 h after CIK cell transfusion. Headache and arthralgia were noted in 2 patients each, and insomnia and asthenia in 1 patient each. All patients recovered within 36 h without any additional special treatment. In addition, no serious clinical adverse effects were observed. Therefore, we demonstrated that autologous CIK cell transfusions were safe and tolerated in CHB patients.



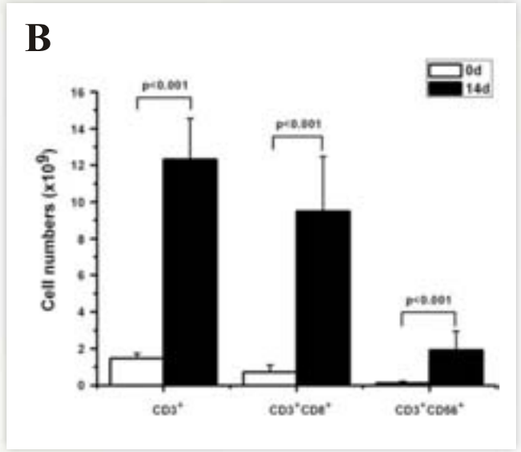


Figure 2
Dynamic changes in the CIK cellular phenotype and proliferation during incubation. (A) Dynamic changes in the proportions of the CIK cell subsets during the incubation period.(B) After 14 d of incubation, the numbers of $CD3^+$,$CD3^+CD8^+$, and$CD3^+CD56^+$cells were significantly increased as compared to their respective baseline levels (P<0.001 in all 3 cases), and the mean expansion in the cell number of $CD3^+$, $CD3^+CD8^+$, and $CD3^+CD56^+$cells were 8.5-, 15.2-, and 16.8-fold, respectively.Control antibodies (eBioscience) for 30 min and were analyzed by flow cytometry as described in our previous report [24].

CIK cell transfusion suppresses HBV replication

During the 6 months before CIK cell treatment, our patients had stable serum HBV DNA load and ALT levels. After CIK cell transfusion, the serum ALT levels and HBV DNA loads decreased (Fig. 4A). While the total virological response rate was 23.8%, it was 0% and 57.1%, in patients with a baseline serum ALT of ≤ 40 U/L and ＞40 U/L, respectively (P< 0.05). Moreover, 4 patients who were biochemical responders with a baseline serum ALT level of ＞ 40 U/L were all virological responders, and 50% of them developed antiviral drug resistance before CIK cell treatment. Our results indicated that better virological and biochemical responses were more frequently encountered in patients with a baseline serum ALT level of ＞40 U/L than in those with a baseline serum ALT level of ≤ 40 U/L, even in the setting of development of antiviral drug resistance. For example, patient 14 (baseline serum ALT level ＞40 U/L) developed drug resistance to lamivudine and received CIK cell transfusions 3 times at successive intervals of 24 and 22 weeks. The decreased serum ALT level and HBV DNA load persisted for＞ 20 weeks since the last autologous CIK cell transfusion and was followed by HBeAg seroconversion (HBeAg−/anti-HBe+)(Fig.4B). Patient 5 (baseline serum ALT level ＜ 40 U/L) received CIK cell treatment 3 times at successive intervals of 12 and 16 weeks. After the first CIK cell transfusion, no significant changes were detected in the serum ALT level and HBV DNA load. When serum ALT increased to＞ 40 U/L, the second CIK cell transfusion was administered, and the patient subsequently exhibited a decrease in the viral load, and a similar effect was found after the third CIK cell transfusion. Patient 17 also had a baseline serum ALT level of ＞ 40 U/L and was resistant to lamivudine therapy. He received CIK cell treatment 2 times at an interval of 24 weeks, which was followed by a reduction in the HBV DNA load that persisted stably for approximately 40 weeks. Patient 21 received CIK cell treatment only once; his baseline serum ALT level was ＞40 U/L, and he also exhibited a similar trend in the decrease in the ALT level and the viral load during the 48-week follow-up.

Moreover, we found that all patients with HBeAg loss or seroconversion after CIK cell treatment had a baseline serum ALT level of ＞ 40 U/L. The rate of HBeAg loss gradually reached 33.3% by the end of the follow-up period. The rate of HBeAg seroconversion peaked at 14.3% at 12, 32, and 36 weeks and then decreased to 9.5% at the end of the follow-up period (Fig.4C). Furthermore, we found that the average number of $CD3^+CD56^+$cells in patients with HBeAg seroconversion ($3.54\pm1.72\times10^9$) was significantly higher than that in patients with non-HBeAg seroconversion ($1.76\pm0.93\times10^9$) (P=0.0169). Therefore, the HBeAg seroconversion correlated with the number of transfused CIK cells. The above results indicate that HBV replication was efficiently suppressed by CIK cell treatment, particularly in patients with a baseline serum ALT level of >40 U/L, even in the setting of antiviral drug resistance.

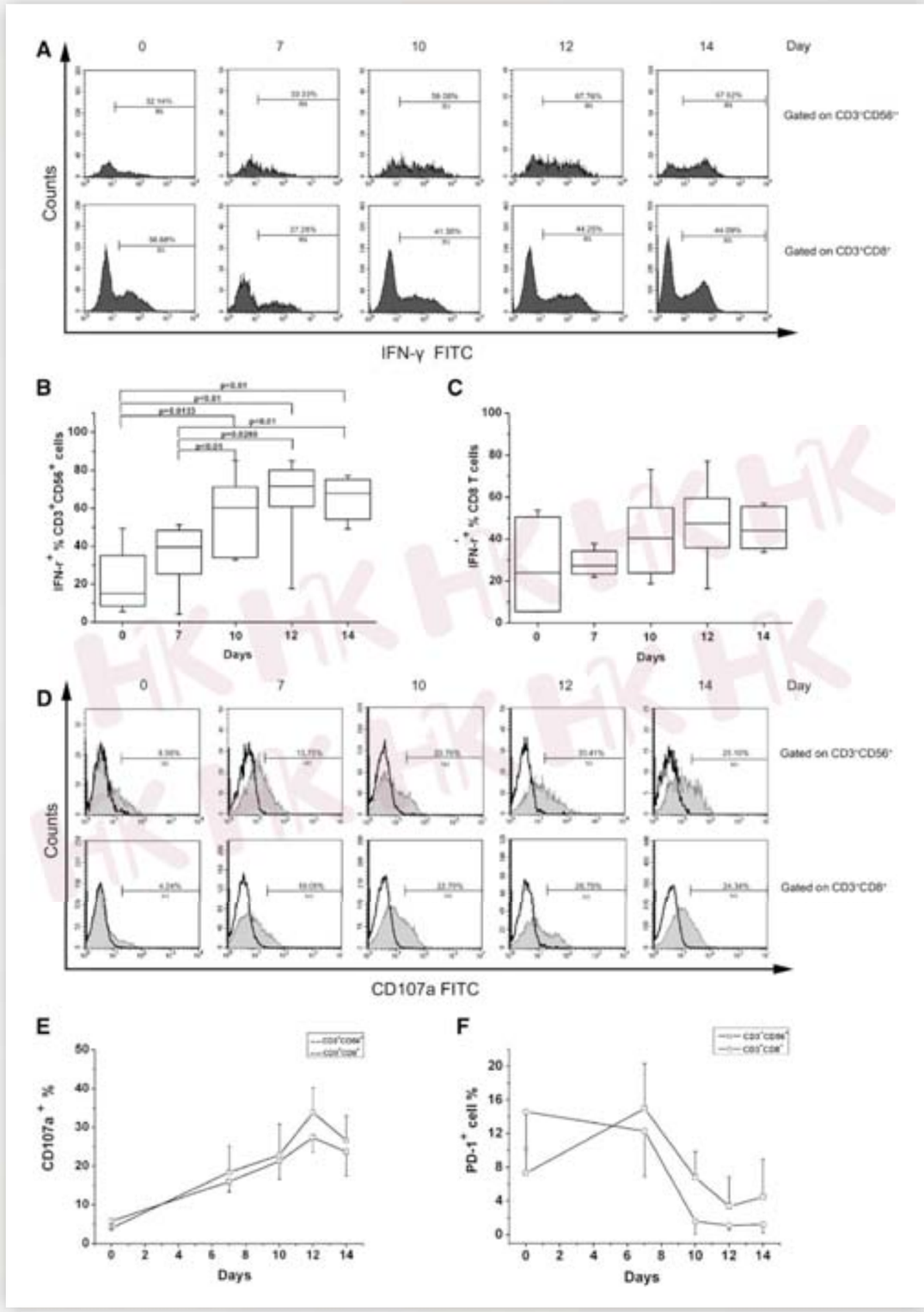


Figure 3
Analysis of IFN-γ-producing and CD107a-expressing $CD3^+CD56^+$ and $CD3^+CD8^+$ cells in the incubation phase. The figure represents the results of IFN-γ-producing cells in a representative patient during the incubation period. (A) The IFN-γ-producing CD^3+$CD56^+$ and $CD3^+CD8^+$ cells were detected by intracellular cytokine staining. (B) During incubation, the percentage of IFN-γ-producing $CD3^+CD56^+$ cells was significantly up-regulated on days 10, 12, and 14 as compared to days 0 and 7. (C) The percentage of IFN-γ-producing $CD3^+CD8^+$ cells showed an increasing trend on culture days 10, 12, and 14. (D) Flow cytometric figures represent thepercentage of positive CD107a-expressing $CD3^+CD56^+$ and $CD3^+CD8^+$ T cells in a representative patient. (E) The curves represent CD107a expression on $CD3^+CD56^+$ and $CD3^+CD8^+$ cells during the 14-d incubation period. (F) The curves represent PD-1 expression on $CD3^+CD56^+$ and $CD3^+CD8^+$ cells during the 14-d incubation period and indicate that PD-1 expression on $CD3^+CD56^+$ and $CD3^+CD8^+$ cells had decreased on days 10, 12, and 14 of the incubation period.

Peripheral $CD3^+CD56^+$ and $CD3^+CD8^+$ cells increased in virological responders

The number of CD3+CD56+ cells transfused back to the patients was much higher in virological responders than in virological non-responders(P=0.0152) (Fig.5A), although CD3+CD8+ cells did not show this trend. In contrast, there was no significant difference between the number of CD3+CD56+ and CD3+CD8+ cells obtained by leukapheresis before CIK cell generation. At the same time, we also found no significant difference in the numbers of other cells (e.g.CD3+CD4+and CD3−CD56+/CD16+) before CIK cell generation (data not shown). The proportion of CD3+CD56+cells in the blood of all patients increased significantly during the first 4 weeks after CIK cell treatment; however,that of the CD3+CD8+ cells increased significantly only during the first 2 weeks after CIK cell treatment (data not shown).In virological responders, the proportion of CD3+CD56+cells significantly increased at week 2 (P=0.0203), 4 (P=0.0142), 8 (P<0.01), and 12 (P=0.0417), and CD3+CD8+cells significantly increased at week 2 (P=0.0262), 4 (P<0.01), and 8 (P=0.0125) of follow- up, respectively(Fig.5B). However, in virological non-responders, only the proportion of CD3+CD56+cells increased significantly during the first 2 weeks of the follow-up period (P=0.0121),while that of CD3+CD8+cells did not show any increase(Fig.5C).CD107a-expression on the CD3+CD56+cells was also significantly up-regulated in the virological responders during the first 8 weeks of the follow-up period (P=0.0290), but only up-regulated during the initial 2 weeks in virological non-responders receiving the CIK transfusion. In addition,CD107a-expression on CD3+CD8+cells was also up-regulated in virological non-responders during 2 weeks of the follow-up period (Figs. 5D and E).After 12 weeks, the proportions of CD3+CD56+ and CD3+CD8+ cells and their CD107a expression were similar to the respective baseline values (data not shown).

Discussion

Strong polyclonal and multi-specific antiviral CD4+-and CD8+-T-cell responses with a type 1 profile of cytokine production play an important role in viral clearance in patients with self-limiting acute hepatitis B and C [25-28]. However, these cellular immune responses are generally weak or even undetectable in patients with chronic HBV or hepatitis C virus (HCV)infection, possibly due to viral replication and persistence[29-32]. The host immune response, including the lack of the CD8+ CTLs that control HBV replication, is significantly suppressed in CHB patients; however, it recovers in a few CHB patients who have been treated with presently available antiviral agents. The suppression of antiviral immune responses in patients with chronic hepatitis B or C infection may, at least in part, be due to the increase in the number of CD4+CD25+regulatory T cells[33,34] which result in impaired CD8+T-cell function[35,36].Correspondingly, the induction of HBV-specific adaptive immune responses has been shown to have limited clinical efficacy in CHB patients. While more clinical trials are required to optimize HBV-specific adaptive immune responses, a new therapeutic modality that activates host immunity is presently being used in patients with CHB.

A controlled design is important to examine the efficacy of CIK cell treatment. In this study, it was an ethical problem to enroll CHB patients in a study who had abnormal serum ALT levels and high HBV DNA loads without use of antiviral drug therapy for 1 year. Therefore, we evaluated the antiviral efficacy of CIK cell treatment by conducting comparisons with the pretreatment status. In the enrolled patients, the HBV DNA load was stably maintained for 6 months before CIK cell treatment. Therefore, we could attribute any observed decrease in the HBV DNA load after CIK cell transfusions to the antiviral function of the treatment. In this study, we prepared the activated forms of immunocytes, i.e., CIK cells, and showed that adoptive transfer of CIK cells is safe and capable of controlling HBV replication and all eviating liver damage in patients with the immune clearance status of CHB. The HBV-specific T cells were undetectable in CIK cells, suggesting that the anti-HBV role of the CIK cell transfusion is not likely to depend on viral specific CD8+ T cells. CIK cells may exert a direct cytotoxic effect through the exocytosis of granules containing perforin and granzyme A/B, which is not dependent on the MHC-restricted mechanism. Moreover, these cells have an indirect cytotoxic effect through the secretion of IFN-γ and tumor necrosis factor alpha (TNF-α) to suppress HBV replication. The functional capacity of CIK cells may be related to the expansion and functional improvement of major effector cells, CD3+CD56+ cells, which comprise only approximately 15% of the peripheral blood [37]; however, culture with the cytokine cocktail resulted in a 10-100-fold increase in the number of these cells. In our study, the number of CD3+CD56+ cells transfused back to the patients was much higher in virological responders than virological non-responders. However, the numbers of other cells, such as CD3+CD8+, CD3+CD4+, and CD3−CD56+/CD16+ (NK) cells did not show this trend. We also found that virological responders who received the transfusion of CIK cells exhibited an elevated proportion of CD3+CD56+ and CD3+CD8+cells in peripheral blood,which may be associated with the boosting of anti-HBV response in these patients.

To our knowledge, this is one of the first studies on the use of CIK cells in nonmalignant patients, although, we and others have reported the efficacy of these cells in HCC patients [23,38]. Accordingly, the main concern was the safety of CIK

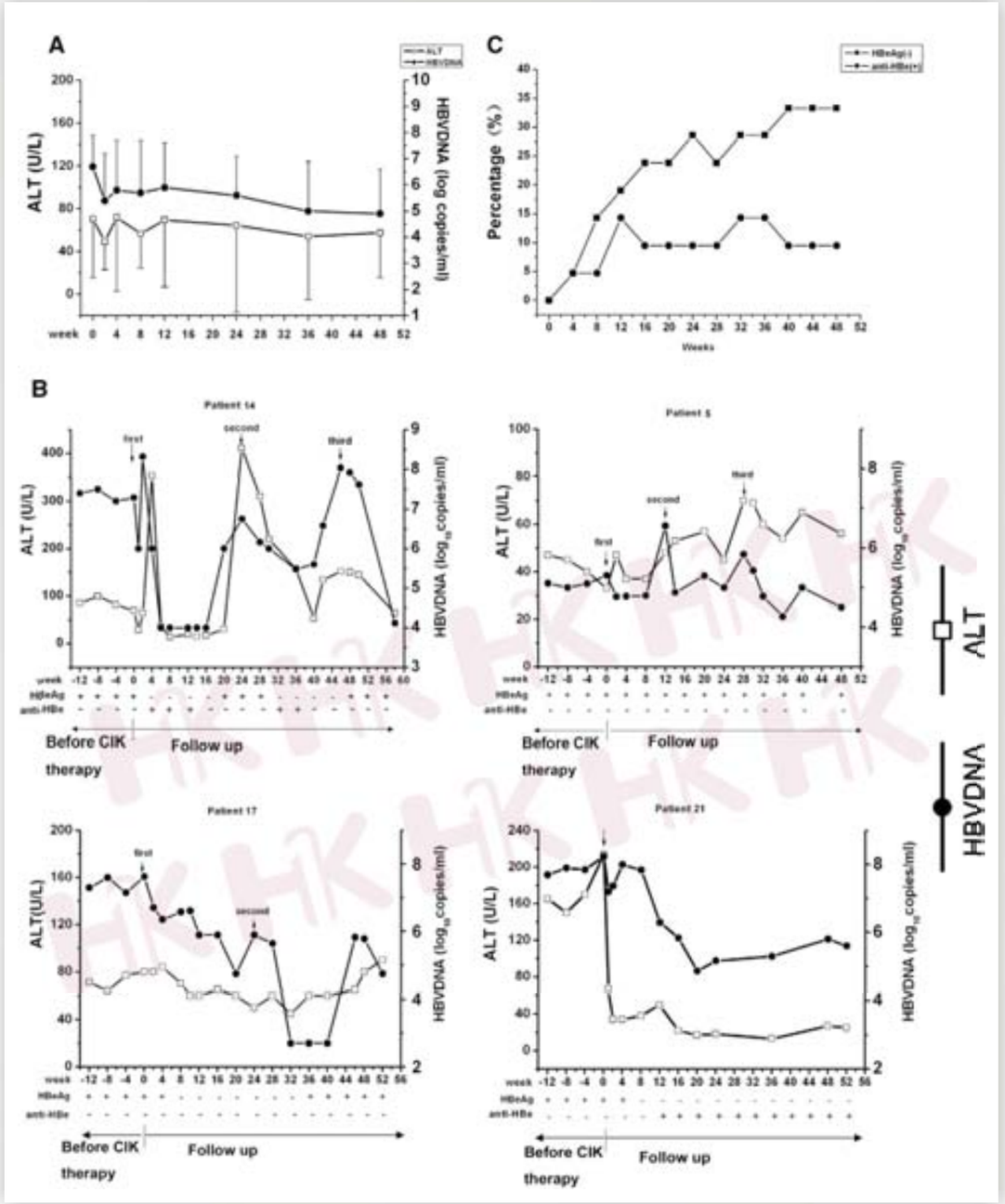


Figure 4
Biochemical and virological responses after autologous CIK cell treatment. (A) The biochemical and virological responses of all patients in the follow-up period after CIK cell treatment. (B) The biochemical and virological responses in the enrolled patients 14, 5, 17, and 21. Patient 14 (baseline serum ALT level, >40 U/L) was subjected to CIK cell treatment 3 times; patient 5 (baseline serum ALT level, ≤40 U/L) was subjected to CIK cell treatment 3 times; patient 17 (baseline serum ALT level, >40 U/L) was subjected to CIK cell treatment 2 times; and patient 21 (baseline serum ALT level, >40 U/L) was subjected to CIK cell treatment only once. (C) The rates of HBeAg loss or HBeAg−/anti-HBe+seroconversion in total patients during the follow-up period. The findings indicate that CIK cell treatment is more suitable for CHB patients having baseline serum ALT levels of >40 U/L than those with ≤40 U/L.

cells in CHB patients. We observed that administration of the CIK cells caused reduced replication of HBV and transient ALT elevation in some patients. Moreover, we did not observe any serious adverse effects in any of the patients. CIK cell therapy appears to be a more beneficial therapeutic option for patients with baseline serum ALT levels of >40 IU/L. This was true for patients who had developed antiviral drug resistance. All patients who were biochemical responders were also virological responders.All patients with HBeAg loss or those who showed seroconversion had baseline serum ALT levels of >40 U/L. Our data suggest that CIK cell treatment was suitable for patients with baseline serum ALT levels of >40 U/L,which occurs during an immune active phase. Therefore,the efficacy of CIK cell treatment for CHB patients may be dependent on the host immune status. These findings provide pertinent information for selection of an optimal therapeutic strategy for patients with the disease in the future.

It is well known that CD107a serves as a functional marker of cytotoxic cells that release their granule components, which include mainly perforin, granzyme A, and granzyme B, through their degradation [39]. In this study, we found that CD107a expression was up-regulated on CD3+CD56+ and CD3+CD8+cells on days 10, 12, and 14, which favors the enhancement of their cytolytic activity against the target cells.

In addition, we found that the percentage of IFN-γ -producing CD3+CD56+ cells was much higher on culture days 10,12, and 14. PD-1 serves as an inhibitory molecule. Its up-regulation can mediate virus- specific CD8+ T-cell exhaustion, whereas blockade of the PD-1/PD-L1 pathway can, at least in part, restore virus-specific CD8+T-cell function and reduce the viral burden[40,41]. Our previous findings suggested that PD-1 ligand (B7-H1) up-regulation on myeloid dendritic cells significantly suppressed T-cell function in CHB patients [42]. Interestingly, in this study, PD-1 was stably down-regulated on the CIK cells, which may, in part, explain the enhanced function of the CIK cells[43-45].The above findings suggest that CIK cell transfusion on culture days 10, 12, and 14 will favor their cytotoxic function against HBV. The proportions and function of CD3+CD56+and CD3+CD8+cells in the peripheral blood of the virological responders improved, which, to some extent, may explain why CIK cell treatment was more effective in these patients.

In conclusion, CIK cells can effectively be generated from CHB patients, and administration of autologous CIK cells is not only safe and tolerated but also efficacious for some patients with active hepatitis B, even if antiviral drug resistance has developed. This protocol represents an alternative immune therapeutic strategy for the disease. Further studies on the use of this approach are warranted in the search of better therapeutic modalities for chronic HBV infection.

Acknowledgments

This work was supported by grants from the National Key Basic Research Program of China (No.2007CB512805), the National Natural Science Foundation of China (No.30571738) and the Hong Kong Research Grant Council (HKU 7679/06M).

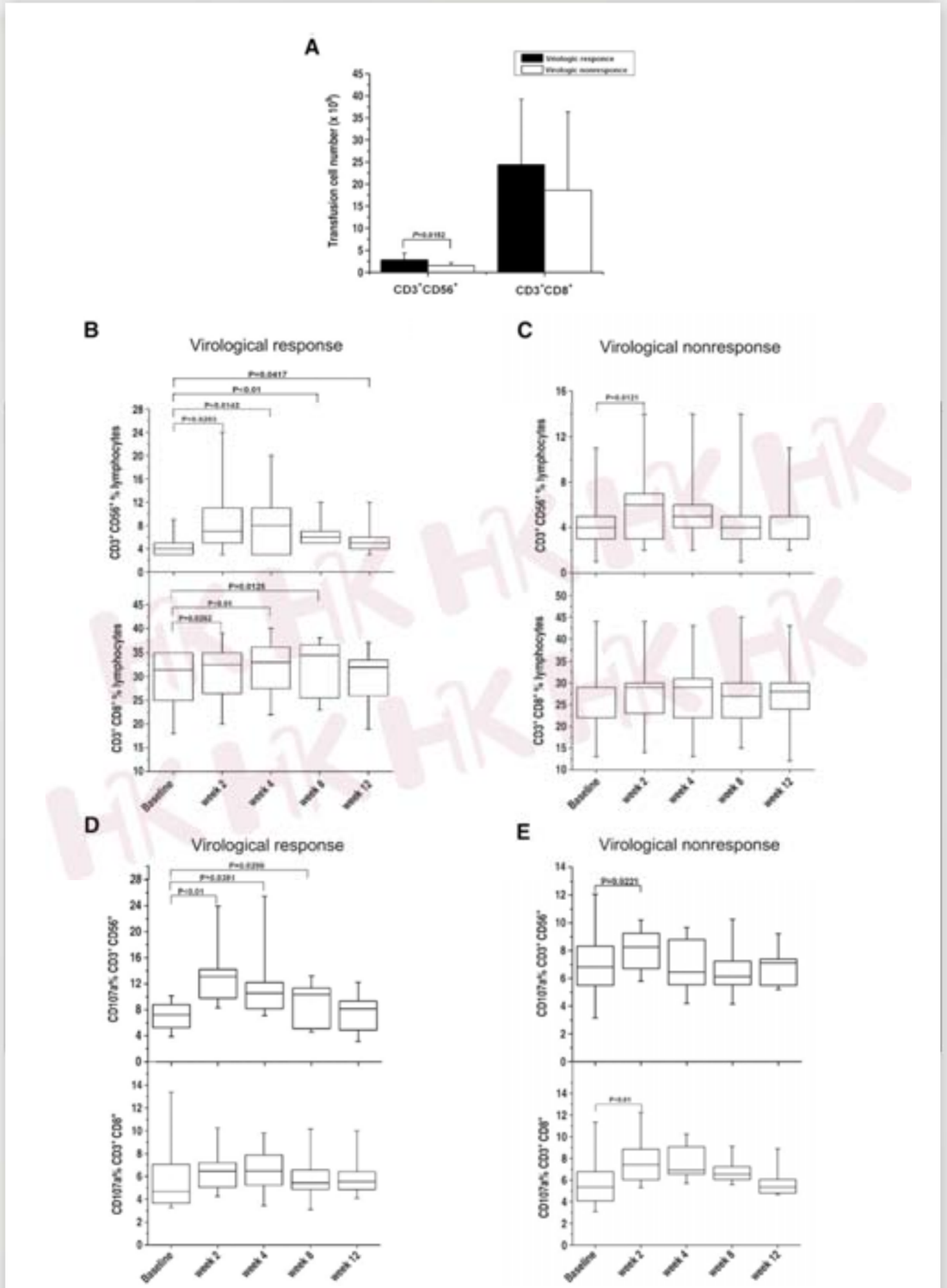


Figure 5

Alteration in the percentages of CD3+CD56+ and CD3+CD8+cells in the peripheral blood after CIK cell treatment. (A) The quantity of CD3+CD56+and CD3+CD8+cells transfused back into the patients who were virological responders and non-responders.(B) The percentages of CD3+CD56+and CD3+CD8+cells in the peripheral blood of the virological responders increased significantly in the first 12 and 8 weeks, respectively, after CIK cell transfusion. (C) In the peripheral blood of the virological non-responders,the percentage of CD3+CD56+cells rather than that of CD3+CD8+cells increased significantly during the first 2 weeks after CIK cell transfusion. (D) CD107a expression on the CD3+CD56+cells in the peripheral blood of the virological responders was significantly up-regulated after CIK cell transfusion. (E) The percentage of CD107a-expressing CD3+CD56+and CD3+CD8+cells in the peripheralblood of the virological non-responders increased significantly only within the first 2 weeks after CIK cell transfusion.

A randomized, controlled trial of postoperative adjuvant cytokine-induced killer cells immunotherapy after radical resection of hepatocellular carcinoma

Hui Dong, Qiang Li *, Jian Wang, Ti Zhang, Da-Lu Kong

Department of Hepatobiliary Surgery, Cancer Hospital of Tianjin Medical University, Tianjin, People's Republic of China
Received 24 July 2007; accepted 21 April 2008
Available online 25 September 2008

CIK过继 免疫细胞治疗肝细胞癌根治性切除术后的随机对照研究

摘要

背景：由于对传统放化疗耐药，肝细胞癌在根治性切除后存在很高的复发率，过继性免疫治疗成为肝癌极富前景的一种治疗方法。

目的：评价CIK过继免疫细胞治疗对肝癌术后的预后。

患者和方法：2000至2002年，共收治127名符合纳入标准的患者，随机分为3组。肝癌术后，41名患者行3疗程CIK细胞治疗（CIK Ⅰ组），43名患者行6疗程细胞治疗（CIKⅡ组），另43名患者术后不接受过继免疫细胞治疗（对照组）。比较三组1、3、5年无病生存期和总体生存率。

结果：log-rank 检验示CIK Ⅰ组(P = 0.001)、CIKⅡ组(P = 0.004)无病生存期显著高于对照组。CIK Ⅰ组、CIKⅡ组组间未见统计学差异(P = 0.345)。cox回归分析示治疗方式是复发与否的一个风险因素。三组总体生存率未见统计学差异。

结论：肝癌术后CIK免疫细胞治疗能预防复发和转移。但对总体生存率未见改善。

Introduction

Through out the world, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death [1,2]. HCC is particularly prevalent in China because of the high infection rate of hepatitis B. Mean while, its incidence is also increasing in the Western world [3]. Despite remarkable improvements in therapeutic procedures of HCC in the past decades, liver resection and liver transplantation are still considered as the potentially curative treatments for HCC. Due to the limitations of liver transplantation (graft availability, selection criteria, and cost), a large number of HCC patients in China were treated with liver resection. However, postoperative recurrence is frequently observed. Therefore, the prevention of recurrence constitutes one of the most challenging issues in improving the efficacy of surgery. As is well known, HCC is resistant to conventional chemotherapy, and is rarely amenable to radiotherapy [4]. We hypothesized that adjuvant immunotherapy might benefit patients who underwent resection of HCC. Cytokine-induced killer (CIK) cells are a unique population of cytotoxic T lymphocytes (CTL). They are non-MHC-restricted and are generated by incubation of peripheral blood lymphocytes with anti-CD3 monoclonal antibody, IL-2, IL-1 and interferon gamma (IFN-γ) [5]. CIK cells represent strong anti-tumor cytotoxicity in vitro and in vivo [6]. These cells have demonstrated higher proliferative and cytolytic activities in comparison with the lymphokine activated killer cells (LAK cells) that are essentially activated natural killer (NK) cells. Accordingly, we conducted a randomized controlled trial to evaluate the efficacy of adjuvant immunotherapy with CIK cells after radical resection of HCC.

Materials and methods

2.1. Patients

According to the selection criteria (Table 1), 127HCC patients who underwent curative hepatic resection in Cancer Hospital of Tianjin Medical University between January 2000 and January 2002 were enrolled in this trial. The nature of this study was fully explained to these patients, and informed consent was obtained from all of them. The study protocol conformed to the principles of Declaration of Helsinki (1983) and was approved by Tianjin Anti-Cancer Association.

2.2. Study design

The aim of this trial was to evaluate the clinical effect of two different courses of adjuvant CIK cells immunotherapy after radical resection for HCC. These 127 eligible patients were randomly assigned into 3 groups. Random drawing of lots and a single-blind method were employed. Posoperative CIK immunotherapy was administered for 3 courses in 41 patients (CIK-I group) and 6 courses in 43 patients (CIK-II group). The other 43 patients didn't receive any post-operative adjuvant therapy (the control group). Among the three groups, no statistical significance was found in patients' age, gender, α-fetoprotein (AFP) level, HBsAg, tumor size, Child-Pugh stage, tumor differentiation, liver cirrhosis, portal hypertension, vascular invasion, blood loss, and time of liver ischemia (Table 2).

Patient selection criteria Table 1

Inclusion criteria	Exclusion criteria
Solitary tumor	Refused to participate
No preoperative transfusion	History of previous treatment
HCC confirmed by pathology	Alcohol abuse after operation
Resection margin >1 cm	Suffer other malignant tumors and/or immunosuppressive diseases
No tumor fracture and hemorrhage	Hypersusceptibility and/or adverse effect
No tumor distant metastases	

2.3. Surgical procedures

All surgeries were performed by the same group of doctors, with resection margin >1 cm. Pringle's manoeuvre was used to control bleeding.

2.4. CIK cells preparation and therapeutic regimen

CIK cells were prepared according to Schmidt-Wolf et al.'s [6] description. Briefly, for in vivo treatment, patients' peripheral blood mononuclear cells (PBMCs) were collected by CS-3000 Plus blood cell separator. For in vitro test, CIK cells were prepared by ficoll separation. The cells were incubated in CM, and rhIFN-γ (1000 U/mL) was added on d 0. After 24 h of incubation, monoclonal anti-body (mAb) against CD3 (50 μg/L), rhIL-1α (100 U/mL) and rhIL-2 (1000 U/mL) were added. Cell density was about 1×10^6 mL^{-1}. Fresh CM with IL-2 was used to replace the old CM every 3 days. Cell phenotypes were identified by FCM on d 0, 7, 14, and 25. Cytotoxicities to target were determined after d 14. All of the above procedures were performed in the immunology laboratory of Cancer Hospital of Tianjin Medical University. CIK Cells were initially transfused back to the patients of CIK-I group and CIK-II group four weeks after operation. With an interval of 2 weeks, CIK-I group received three courses of treatment and CIK-II group received six courses of treatment. The number of CIK cells transfused back to patients ranged from1.0×10^{10} to 2.0×10^{10}per course.

2.5. Follow-up method

All patients were followed up at the outpatient clinic every month from the date of initial treatment up to January 2007, or up to the time of death. Abdominal and pelvic ultrasonography and chest X-ray were the routine examinations. When recurrence/metastasis was suspected, further evaluations were made by abdominal CT scan and, if necessary, by ultrasound-guided biopsy to confirm the diagnosis.

2.6. Statisticalanalysis

The overall survival and disease-free survival rate were calculated by Life Table. The KaplanMeier method was used to plot the estimated survival curves and disease-free survival curves. We used log-rank test to identify the association factors of disease-free survival rate. These factors then underwent multivariate analysis by Cox's proportional hazards model. A P–value < 0.05 was considered statistically significant. Data were analyzed by SPSS Version 13.0 for Windows.

Results

3.1. Side effects

Due to side effects, three patients in CIK-I group and two patients in CIK-II group failed to fulfil the CIK immunotherapy. Persistent febrility was the only side effect observed in the above five patients. However, the temperature of all these patients was lower than 38.5 °C. During the 5-7 years' follow-up, no long-dated side effects had been observed in all patients of the CIK-I and CIK-II groups.

3.2. Follow-up results

In CIK-I group, 27 patients suffered HCC recurrence, 5 patients suffered HCC metastasis, 3 patients suffered HCC recurrence and metastasis, and 29 patients died (1 patient died of pulmonary artery embolism, 6 patients died of esophageal veins hemorrhage, and 22 patients died of advanced HCC). While in CIK-II group, 30 patients had HCC recurrence,6 patients had HCC metastasis, 1 patient had HCC recurrence and metastasis, and 31 patients died (1 patient died of myocardial infarction, 2 patients died of pulmonary artery embolism, 5 patients died of esophageal veins hemorrhage,and 23 patients died of advanced HCC). In the control group,30 patients suffered HCC recurrence, 7 patients suffered HCC metastasis, 3 patients suffered HCC recurrence and metastasis, and 32 patients died (1 patient died of cerebral infarction,2 patients died of pulmonary artery embolism, 6 patients died of esophageal veins hemorrhage, and 23 patients died of advanced HCC).

3.3. Survival rate and disease-free survival rate

The 1-, 3- and 5-year disease-free survival rates were83.1%, 31.7% and 23.3% in CIK-I group; 84.7%, 30.5%, and 19.4% in CIK-II group; and 82.6%, 20.9% and 11.2% in the control group, respectively. The KaplanMeier method was used to estimate the disease-free survival rates in the 3 groups.

Table 2
Clinical and pathological features of the 127 patients

Characteristics	CIK-I group	CIK-II group	Control group	X^2	*P*-Value
Gender					
Male	31	32	34	0. 278	0. 870
Female	10	11	9		
Age					
≥50 years	27	26	28	0. 314	0. 855
<50 years	14	17	15		
HBsAg					
Positive	32	33	31	0. 450	0. 798
Negative	9	10	12		
Liver cirrhosis					
Yes	34	34	33	0. 501	0. 778
No	7	9	10		
Child-Pugh classification					
Child-Pugh A	34	34	34	0. 261	C. 878
Child-Pugh B	7	9	9		
Portal hypertension					
Yes	24	26	27	0. 160	C. 923
No	17	17	16		
Tumor size					
≥5 cm	17	19	21	0. 474	C. 789
<5 cm	24	24	22		
AFP					
Positive	33	34	33	0. 180	C. 914
Negative	8	9	10		
Vascular invasion					
Yes	17	19	23	1. 335	C. 508
No	24	24	20		
Tumor differentiation					
Well differentiated	8	10	9		
Moderately differentiated	19	20	19	0. 335	C. 987
Poorly differentiated	14	13	15		
Blood loss					
≥500 ml	8	11	13	1. 147	0. 564
<500 ml	33	33	31		
Time of liver ischemia					
≥20 min	12	14	13	0. 134	0. 935
<20 min	29	29	31		

The log-rank test showed significantly higher disease-free survival rates in CIK-I group (P= 0.001) and CIK-II group (P = 0.004) than in the control group, but no statistical significance was found between CIK-I group and CIK-II group (P= 0.345) (Fig. 1). Through univariate analysis, 7 of the 13 variables were identified as association factors of disease-free survival rate (P < 0.05, Table 3). Multivariate analysis of the 7 showed that treatment modalities (different courses of CIK cell therapy), liver cirrhosis, tumor size, tumor differentiation and vascular invasion were significant factors influencing the disease-free survival of HCC patients (P < 0.05, Table 4). The 1-, 3- and 5-year overall survival rates were 87.3%, 66.7% and 37.9% in CIK-I group; 86.7%, 63.8% and 38.1% in CIK- II group; and 87.1%, 65.2% and 36.9% in the control group, respectively. There was no statistical significance among the three groups (P=0.884, Fig. 2). Despite no significant differences in the factor of liver cirrhosis among the three groups, we still performed a separate analysis for cirrhotic patients alone in order to prevent bias. The results suggested no statistical significance in survival rate among the three groups. However, the disease-free survival rates of CIK-I and CIK-II groups were higher than that of the control group. No statistical significance was detected in disease-free survival rate between CIK-I group and CIK-II group.

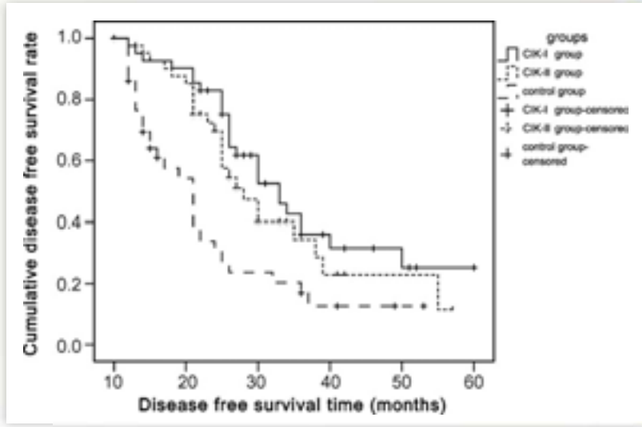


Fig. 1.
Disease-free survival curves of the three groups. KaplanMeier was employed to estimate the disease-free survival rates of the three groups. Log-rank test shows a significantly higher rates in the CIK-I group (P = 0.001) and the CIK-II group (P = 0.004) than in the control group. No statistical significance was found between the CIK-I group and the CIK-II group (P = 0.345).

Discussion

In spite of the great improvement in hepatic operation technique and early diagnosis of HCC, the prognosis of HCC is still poor because of a high incidence of postoperative recurrence and metastasis. After curative resection or transplantation, tumor recurrence rate can be as high as 25% per year [7]. Although some centers have reported excellent long-term results, survival after hepatic resection or transplantation is as low as 50% at 3 years and 20–30% at 5 years [8,9]. Systemic and regional chemotherapy or chemoembolization have been largely demonstrated of little benefit for patient survival in randomized trials [10,11]. Thus, many other adjuvant therapies have been employed to lower HCC recurrence rate and improve long-term survival rate. Some of these modalities have exhibited promising benefits in individual trials. For example, interferon therapy appears to decrease recurrence rate after resection of hepatitis C virus or hepatitis B virus-related HCC in some randomized controlled trials [12,13]. Some retrospective studies found that pre- and postoperative adjuvant TACE could prolong the survival of patients with risk factors and improve disease-free survival after hepatectomy [14,15]. Other adjuvant modalities, such as oral acyclic retinoid acid [16] and intra-arterial radioiodine therapy [17], have also presented with some promises in the treatment of HCC.

Table 3
Factors associated with disease-free survival rate identified by univariate analysis

Factors	X^2	*P*-Value
Gender	2.840	0.092
Age	1.238	0.266
HBsAg	0.441	0.507
Liver cirrhosis	6.272	0.012
Child-Pugh classification	4.817	0.028
Portal hypertension	1.635	0.387
Tumor size	11.593	0.001
AFP	1.574	0.217
Vascular invasion	9.587	0.007
Tumor differentiation	5.354	0.023
Blood loss	3.281	0.042
Time of liver ischemia	2.096	0.136
Treatment modalities	15.667	0.001

Table 4
Factors influencing disease-free survival rate identified by multivariate analysis

Factors	β	S.E	*P*-Value
Treatment modality	0.374	0.273	0.041
Liver cirrhosis	0.423	0.214	0.034
Tumor size	0.447	0.116	0.021
Tumor differentiation	0.552	0.103	0.013
Vascular invasion	0.435	0.161	0.026

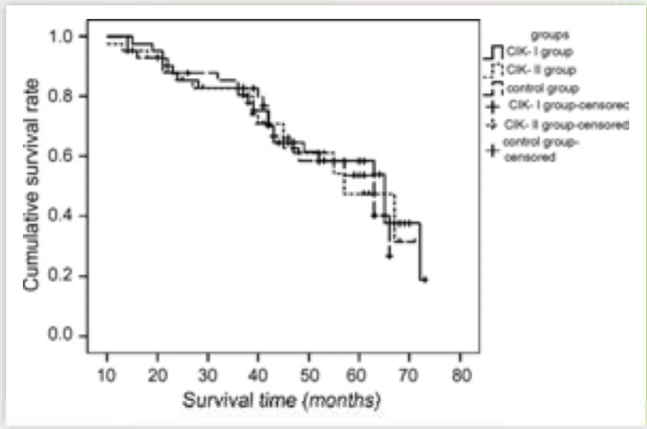


Fig. 2.
Survival rate curves of the three groups. Kaplan–Meier was employed to estimate the survival rates of the three groups. Log-rank test shows no significant difference among the three groups ($P = 0.884$).

With the rapid advance of molecular biology technology, the application of cytokines is thought to be a promising strategy for cancer treatment. Cytokine immunotherapy has many advantages compared with traditional therapeutic methods such as chemotherapy and radiotherapy. Cytokine immunotherapy not only has fewer side effects, but also can avoid tumor dysimmunity and specific tolerance of tumor antigen. LAK cells adoptive immunotherapy is a classical therapeutic method for many malignant tumors including HCC. However, its role in the prevention of HCC recurrence remains controversial [18-20]. Stanford University School of Medicine first reported that CIK cells had a strong antiproliferative capacity and cytotoxicity on tumor cells [21]. Further studies have demonstrated that CIK cells, which are lymphocytes induced by multiple cytokines, have better anti-tumor effects compared with LAK cells (lymphcytes activated by IL-2 alone) [22]. In addition, CIK cells have minimal cytotoxicity against normal hematopoietic cells and do not cause myelosuppression. Therefore, the therapy with CIK cells has great prospects in cancer treatment and is catching more attention [23]. Importantly, application of CIK cells has approved beneficial for some patients with malignant tumors in some clinical trials [24,25]. As for HCC, the toxicity of CIK cells has been testified in a few in vitro studies. However, there are few documents concerning in vivo studies on the effect of CIK cells. Our data demonstrated that CIK immunotherapy improved disease-free survival rates but had no effect on survival rates. Meanwhile, no significant difference was found in survival rates and disease free survival rates between the 2 CIK groups. We presume that the early three courses of CIK immunotherapy play an important role in controlling tumor recurrence. In this trial, the first course of adjuvant CIK immunotherapy was given 4 weeks after radical resection of HCC. We hypothesize that early CIK immunotherapy after operation may be the most beneficial. Although there were no statistically significant differences among the three groups in the five factors which influenced disease-free survival rates, the control group showed a slightly higher percentage of cirrhosis and vascular invasion. This may cause bias and thus may affect the conclusion. In order to solve this problem, a larger scale trial should be conducted. Regarding to the influence of route of administration and dosage of CIK cell therapy, we will further carry out corresponding clinical trials.

Conclusion

Postoperative adjuvant immunotherapy with CIK cells may be a valuable therapeutic strategy for HCC patients in preventing recurrence and metastasis, although it has not approved effective on the overall survival in this trial. Log-rank test shows no significant difference among the three groups ($P = 0.884$).

免疫细胞相关临床试验

编者按：
据NIH统计，目前世界上已经进行上千个免疫细胞相关临床试验，编者每期将介绍近期更新的部分临床试验信息，尤其是跟合一康生物科技有限公司合作的科研机构的临床研究信息，如有特别需要，请联系合一康生物学术部。

* Study of Chemotherapy With Adoptive Cellular Therapy With DC-CIK Cells in Triple Negative Breast Cancer Patients (DCCIK)
  Status: **Recruiting**
  Condition: Breast Neoplasms/Neoplasm Metastasis
  Sponsor: Beijing Cancer Hospital
  ClinicalTrials.gov Identifier: NCT01395056
  Estimated Enrollment: 50
  Study Start Date: July 2011
  Estimated Primary Completion Date: December 2013 (Final data collection date for Primary outcome measure)

* Laboratory-Treated T Cells With or Without Ipilimumab in Treating Patients With Metastatic Melanoma
  Status: **Recruiting**
  Condition: Recurrent Melanoma/Stage IV Melanoma
  Sponsor: **Fred Hutchinson Cancer Research Center**
  ClinicalTrials.gov Identifier: NCT00871481
  Phase: Phase I/Phase II
  Estimated Enrollment: 20
  Study Start Date: February 2009
  Estimated Primary Completion Date: February 2012 (Final data collection date for Primary outcome measure)

* DC Vaccine Therapy Combined With Cytokine-Induced Killer Cell in Treating Patients With Renal Cell Carcinoma
  Status:recruiting
  Condition: Renal Cell Carcinoma
  Sponsor: Fuzhou General Hospital
  ClinicalTrials.gov Identifier: NCT00862303
  Phase: Phase I/Phase II
  Estimated Enrollment: 100
  Study Start Date: March 2009
  Estimated Primary Completion Date: June 2015 (Final data collection date for primary Outcome measure)