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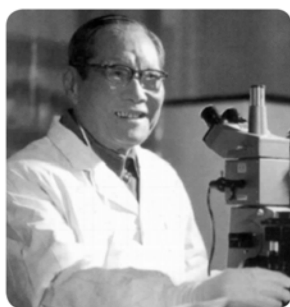
中国生理学会

编者按：2011 年，中国生理学会成立 85 周年之际，学会编辑出版了以王晓民理事长为主编的上下两本图书，上册为《根深叶茂 蔚然成荫——中国生理学人物记》，下册为《根深叶茂 蔚然成荫——中国生理学团队记》。从 2013 年第 3 期开始，《生理通讯》将陆续转载，以飨读者。

生理学家蔡翘

范 明



蔡 翘
（1897年-1990年）

蔡翘，字卓夫，族名义忠。1897 年 10 月 11 日生于广东省揭阳县新墟（亨）镇仙美村。蔡翘七岁起在本村私塾和镇上兰田小学读书。1917 年潮安县金山书院毕业，1918 年赴复旦大学附中补习英文并到北京大学中文系当旁听生。

1919 年秋，他胸怀“科学救国” 志从上海坐船到美国留学。他在加利福尼亚大学用两年时间学习完大学心理学课程。1922 年入芝加哥大学生理系读研究生。期间曾在生物学图书馆勤工俭学。他与吴有训、周培源、潘菽、杨武之（杨振宁之父），袁敦礼、张锡均等曾为室友。学习期间工作发表4篇论文，在袋鼠“顶盖前核”（nucleus pretectalis）的研究中，他发现了在间脑和中脑之间有一未被描述过的以小细胞为主的神经核团。后人证明其功能意义远远超出视觉系统的范围。因而被称为“蔡氏区”，以示不忘他的贡献。1924 年发表博士论文“大白鼠的记忆曲线”并因学业优秀获芝加哥大学金钥匙奖。

1925年回国任复旦大学教授。1926年加入新成立的中国生理学会。1927年秋受聘于上海中央大学医学院，编著了50章，70万字的我国第一本中文《生理学》教科书（1929年，商务印书馆出版）。培养出一批我国早期的生理学工作者，如冯德培、童第周、徐丰彦、朱鹤年、沈霁春和蒋天鹤等。期间阐明了甲状旁腺切除后肌肉抽搐以至死亡的主要原因是血钙浓度严重下降。还揭示了肝通过糖原合成与分解，在保持血糖正常浓度中的作用和机制。

1930年秋美国洛氏基金会资助，赴英国和德国进修，先在伦敦大学著名生理学家伊文思（C.L Evans）教授实验室从事糖代谢的研究，观察乙醚、amytal等麻醉剂的影响，及动物在断头和去大脑之后肝糖原的恢复过程。继而在剑桥大学著名神经生理学家，诺贝尔奖金获得者阿德里安（E.D. Adrian）教授实验室研究麻醉剂，主要是可卡因对蛙趾单条神经纤维动作电位传导的影响。有关研究成为感受器适应现象的经典案例而被写入多本专著。1931年冬在德国法兰克福大学和其他大学的著名的生理学实验室做短期访问。1932年春回国，继续在上海“中央大学”医学院任教。1937年1月到南京“中央大学”医学院任生理学教授。主要开展脾脏与红细胞渗透性的关系及其影响因素和机制的研究；关于溶血物质与抗溶血物质的研究；关于血

清中缩血管物质的研究；关于止血机制的研究，以及关于我国人各种生理水准的调查统计研究等。

抗日战争期间学校内迁成都，他与郑集、童第周等人成立中国生理学会成都分会，组建了生理学研究所。主要助手朱壬葆、周金黄、吴襄、徐丰彦、李瑞轩、匡达人、易见龙、林春猷、蔡纪静等，招收4名研究生（宋少章、程治平、陈定一、李昌甫），接收十几名进修生，如李瑞轩、濮璃、尤寿山、孟宪章、陈兆仁、张培棪、杨浪明、邱琼云等。其中有延安军医学校秘密送来的同志。还曾经向延安提供自制的生理仪器。在此期间，曾经在日寇飞机轰炸间隙继续实验研究。

1943年夏，作为交换教授与费孝通等六人应邀赴美讲学与研究一年，在芝加哥讲演时，介绍我国抗日战争情况，呼吁国际社会援华。他们的讲演稿一起被编成《来自中国的声音》在美国专册出版。这一年中，蔡翘在哥伦比亚大学医学院与美国学者合作继续研究血管中的缩血管物质问题，取得一定成果，对后来5－羟色胺（血管紧张素）的发现起到了重要作用。

1948年他代理“中央大学”医学院院长，并被选为“中央研究院”院士。

新中国成立后，他任南京大学医学院院长。1952年任第五军医大学校长。1954年遵照中央军委命令调入军事医学科学院，任副院长和院学术委员会主任。1957年，军事医学科学院成立军事劳动生理研究所，他兼任所长。1955年被选为中国科学院学部委员。曾被选为第一至第五届全国人大代表，1961年加入中国共产党。1964年—1981年任中国生理科学会（含生理、生化、药物、病理生理、生物物理和营养六个学科）理事长。1981年辞去理事长职务，被中国生理学会理事会推举为名誉理事长。

蔡翘教授在新中国成立后主要从事并领导特殊环境生理学的研究，是我国军事劳动生理学、航空航天医学和航海医学的创始人。他编写了《航空医学入门》，建成我国独特的钢筋水泥低压舱、高空减压舱、爆炸减压舱、动物和人体用的加速度离心机、地面弹射救生装置、模拟失重装置、航海研究所用的潜水加压舱，以及高温低温舱等多项大型设备，并利用这些设备进行了大量研究工作。这不仅测定了国人各方面的重要生理数据，而且根据这些数据提出了一系列适合我国情况的防护制度和装备要求，如航空加压控氧制度，潜水减压制度，抗荷服、代偿服和潜水服的生理数据要求及生理性能鉴定方法等。在短短数年内，为我国年轻的航空航海医学工作建立了研究基地，提供了一整套研究条件，明显地缩小了我国在这些研究领域与国际先进水平之间的差距，通过这些工作也培养了一批学有专长的科技人员，为我国的航空航海医学研究的发展，奠定了坚实的基础。

“文化大革命”期间他受到迫害，失去了工作条件，被安排打扫厕所。记得他曾经很认真的告诉我：他负责的厕所是全工作区最干净的。他借此时间埋头著书，于1979年出版了六十多万字的《航空与空间医学基础》。1978年，蔡翘教授参加全国科学大会，就座于主席团。他又焕发科研青春，号召“树雄心立壮志，攀登生理科学高峰”，指出“基础医学对实现医学科学现代化的重要性”以及“为解决军事医学问题而努力”。在他的积极建议下，军事医学科学院成立了基础医学研究所，他亲自指导该所神经生物学室的工作并培养了一批研究生。

蔡翘教授自1922年从事生理学工作至逝世，一生艰苦创业，在生理学领域进行了多方面的开创性工作；他涉猎广泛，有过许多重要的发现和成就，先后发表过100多篇学术论文和11本专著，教科书。他勤奋好学，始终如一，

以至耄耋之年仍能敏锐地掌握当代生理科学发展趋势。他生活很有规律，终生坚持锻炼身体，年逾九十仍能坚持学习和科研工作。

蔡翘不仅是一位著名科学家，同样也是一位受人尊敬的教育家。他提携新秀，培养了几代人才，为发展我国生理学和医学教育事业作出了卓越贡献。他曾回忆他的执教经验：一是主张教学与科研结合，身教与言教结合，身教重于言教。二是循循善诱，指明方向，使学生热爱专业。他不仅在科研精神和治学态度方面是学生的榜样，而且在思想品德，生活作风等方面也是学生的模范，处处“为人师表”。蔡翘培养的学生遍布全国，很多成为各大学或研究机构中生理学与相关学科的翘楚。

蔡翘教授一生勤勤恳恳，为改变我国的贫穷落后面貌，为我国生理学事业的发展和培养人才，艰苦卓绝地坚持奋斗数十年如一日。几经创业，几经搬迁，战争和动乱，但毫不气馁，先后在复旦大学，吴淞“中央大学”、南京“中央大学” 创建生物学科和生理学科，创建过生理学、军事劳动生理，航天航空医学、航海医学和基础医学等五个系所。先后发表过100多篇学术论文和11本专著、教科书。硕果累累，培养了几代生理学界科研人才。他不愧为中国现代生理学奠基人之一，医学教育界的一代宗师。

他对我国的科学事业极为关心，衷心拥护党关于发展科学事业的“百家争鸣” 的方针，对于医学、生物学界多次出现的违反“百家争鸣”的方针的倾向和做法，总是挺身而出，坚持真理，提出自己的科学见解。1962年在周恩来总理、聂荣臻副总理领导的广州会议上，就如何发展我国自然科学和对待知识分子问题发表了坦诚的意见。1963年8月又就如何贯彻《关于自然科学研究机构当前工作的十四条意见》写了《关于科研工作的建议信》，得到军委聂荣臻副主席的充分肯定和高度评价。20世纪80年代，对于社会上假借“人体科学”搞的特异功能现象也旗帜鲜明的予以反对。这样的科学家正是中国科学的脊梁。

蔡翘的科学精神和英名与中国生命科学永世长存。

（本文有关内容主要来自吴襄、张香桐先生和蔡雪丽大姐提供的材料，在此谨致谢意。）

Professor Chiao Tsai finished his psychologically training in California University (1919 - 1921), and entered Chicago University as a post-graduate for neuro-anatomy and physiology (1922 - 1925). He started his teaching and research career in Fudan University and National Central University in Shanghai as a professor on physiology (1925 - 1937), and got together Te-Pei Feng, Di-Zhou Tong, Feng-Yan Xu, He-Nian Zhu, Qi-Chun Shen and He-Tian Jiang et al as the lab members. He went to UK and Germany as a senior visit scholar within lab of CL Evans, and later, E.D. Adrian who was winner of Nobel Prize (1930 - 1932).

He continued his research and teaching during War of Resistance against Japan in Chengdu and built up a new institute of physiology with scientists from whole China, even from Yanan. In 1943, he visited USA and published *Voice from China* with other Chinese professors to support homeland.

He was selected as president of Medical College of National Central University and was elected as member of Academia Sinica of China in 1948.

After 1949, he joined PLA and entered Academy of Military Medical Sciences in 1954 as vice president and chairman of Academic Council. From then on, he worked for the army and become the founder of physiology and

medicine of space, aviation and marine in New China. He was elected as member of CAS in 1955 and president of Chinese Association of Physiological Science from 1964 to 1981.

He continued his research even in Culture Revolution and after until his last day. Within his career, over 100 papers and 11 monographs have been published. More over, he fostered many students for Chinese biomedicine, and some of them became founders of special fields of biomedicine in China.

中山大学中山医学院生理学教研室

中山大学中山医学院生理学教研室成立于1953年，当时由著名生理学家、国家一级教授林树模任主任。林树模教授在消化、内分泌生理、血液化学、物质代谢等研究领域取得了卓越的成就，并致力于推动我国生理学界同仁的学术交流工作。他是中国生理学会最早的会员，1951年后历任各届中国生理科学会理事；1955年林教授组织成立了中国生理科学会广东省分会并历任分会的理事长，极大地推动了广东省生理科学的发展。随着中山医学院的发展，先后有一批大师级教授如陈培熹、侯慧存、卢光启、陈毓槐、詹澄扬、潘敬运等在生理教研室从事科研和教学工作。

中山医学院生理学教研室是全国首批博士点和硕士点授权单位、博士后流动站学科点、国家教委国内访问学者培养单位、卫生部生理学教师进修基地、中国生理学会副理事长单位和广东省生理学会理事长单位、中国生理学会教育委员会常务理事单位、普通高等教育“十一五” 国家级规划教材《生理学》主编单位、《生理学》教材（科学出版社）主编单位、《生理学》卫生部规划教材参编单位、《生理学国家试题库》建设主持单位。

教研室现有教职工16人，拥有博士学位者14人。其中教授、博士生导师4名，平均年龄55岁；副教授5名，平均年龄36岁；讲师5名，平均年龄35岁；技师2人。在读博士、硕士研究生32名。其中，二级教授、国家级教学名师1人（王庭槐教授），南粤优秀教师3人。以王庭槐教授为课程负责人的生理学课程是中山医学院唯一的“双首批课程”，即首批“广东省重点课程”和“广东省优秀课程”，2002年入选“中山大学精品课程”，2003入选“广东省精品课程”，2004年入选“国家精品课程”。目前教研室主要面向临床医学八年制、临床医学五年制学生，讲授《医学生理学》理论课程和《生理实验科学》实验课程。同时面向研究生和本科生，讲授《高级生理学进展》、《神经生物学进展》和《生物反馈进展》等课程。

自1989年以来，在王庭槐教授的主导以及全体教师的共同努力下，我们进行了持之以恒、形成系统的教学改革，取得了良好的教学效果，并具有了以下的教学特色：

1）坚持不断用先进的教育理念、学习理念来指导本课程的建设，50年来坚持教授上本科课程已成惯例，系统地、持之以恒不断深化教学改革已成传统。

2）理论课重视启发科学思维，实验课强

调能力培养，特别是在实验教学改革中，在全国率先实行“三合一，跨学科”的方式，建立了新型生理学实验课教学模式，并在全国诸多兄弟院校中得到了应用、推广。

3）以传帮带促进教师队伍的建设，以教材建设推进教学内容建设，以科学研究及时推动教学发展，以现代先进教育技术来提升本科教学的教学力和教学效果，以科学管理和教学监控保证课程建设和教学质量。

4）坚持传承创新，已形成“三基三严、三早两强”（基础理论、基本知识、基本技能；严格要求、严肃态度、严密方法；早期接触临床、早期接触科研、早期接触社会；创新能力强、外语和实践能力强）的教学特色。

近二十年来，生理教研室荣获国家级教学成果二等奖4项，省级教学成果奖8项，校级教学成果一等奖11项；获得各种教学改革基金资助34项，发表教学论文70篇，主编国家级教材《生理学》3本、副主编及参编教材15本。生理学课程自入选国家精品课程以来，充分发挥着辐射示范作用，课程负责人王庭槐教授先后应邀在中国生理学会第八届全国生理学教学研讨会、华南港澳地区高校教育技术协作委员会学术年会、全国第二次高等学校医学教学改革会、教育部全国高校教师网络培训中心生理学青年骨干教师培训会上，积极向同行推广课程建设经验，取得了广泛的辐射效果。近年来课程负责人王庭槐教授率先将国际新型TBL（Team Based Learning）教学法引入到生理学的教学中，取得良好的教学效果。同时应邀到第三届北京国际医学院校长高峰论坛、两岸四地学术交流会等大会，以及包括中山大学各附属医院、暨南大学医学院、首都医科大学、天津医科大学、广西医科大学、广州医学院、台湾中山医学大学、台湾高雄大学等20余所院校进行TBL教学法的专题演讲，积极推广这一新型教学法在国内医学教学的应用。

目前，教研室拥有心血管生理学、神经生理学、生物反馈学三个研究方向的科研实验室。研究领域主要有：性激素的心血管效应及其分子机制的研究、突触可塑与疾病、痛觉病理生理、神经生理与细胞保护、生物反馈的生理机制研究。

附：主要学科带头人简介

王庭槐，男，教授，博士生导师，生理学国家精品课程负责人。

1982年毕业于中山医学院医疗系，毕业后留校任教，历任生理学教研室助教，讲师，副教授、教研室副主任。曾兼任中山医科大学校长助理兼校办主任、中山大学教务处处长、中山医学院（医学部）副院长（副主任）、中山大学医学教务处处长、《家庭医生》杂志社副总编。

现任中山大学医学部副主任、中山大学医学情报所所长、兼任澳门镜湖医院院长，国家级教学名师、国家精品课程生理学课程负责人、国务院有突出贡献的专家津贴获得者、全国高等医学教育学会教学管理研究理事会副理事长、2007—2010年教育部高等学校医药学科（专业）教学指导委员会委员、教育部高等学校医药学科（专业） 教学指导委员会委员、教育部本科教学工作水平评估专家；中国生理学会理事、中国生理学会循环、肾脏生理专业委员会委员、广东省生理学会副理事长兼秘书长、《生理学通报》主编、普通高等教育“十一五”国家级规划教材《生理学》主编。

长期从事生理学的科研和教学一线工作，四次获得国家优秀教学成果奖。建立了以研究性学习为特点的新型生理学实验课教学模式（获1993年国家优秀教学成果二等奖）；提出了“三早”（早期接触临床、早期接触科研、早期接触社会）教育模式，并付诸实施（获2005年国家优秀教学成果二等奖）；实施教育部本

科教学质量工程建设，是两门国家级精品课程（生理学、实验生理科学）的课程负责人及实验生理科学国家级教学团队负责人。

课题组为中山医学院心血管生理学和生物反馈研究室。主要成员有：付晓东副教授（教研室副主任）、向秋玲副教授（中山医学院院长助理）、谈智讲师（实验生理科学副主任）、林桂平博士（实验室技术负责人）。主持国家自然科学基金5项，主持及参与其他省部基金10多项，发表科研论文110多篇，其中SCI收录论文20余篇，培养研究生近三十名。在心血管生理学研究方面，主要从事甾体性激素心血管效应及其信号转导机制的研究，学术成就包括：（1）揭示了雌激素对血管各层作用及其机制，发现了血管内皮细胞上膜雌激素受体的存在及其介导的非基因效应，首次报道了雌激素膜受体的解离常数和最大结合容量。阐明了雌激素心血管效应与eNOS、ERK信号通路、caveolin-1等的关系（本研究获得了2009年教育部自然科学奖二等奖）；（2）发现雌激素可通过上调caveolin-3表达，进而抑制压力超负荷诱导的心肌肥厚，揭示了雌激素保护心肌的部分内在机制；（3）发现雌激素可通过下调MCP-1抑制炎性反应和上调caveolin-1蛋白调节内皮祖细胞（EPC）的增殖，在抗AS中起重要作用；（4）发现了雌激素可以通过ERα／Src／PI3K／RhoA/Rock-2信号转导通路，调控Ezrin蛋白的活性，从而引发乳腺癌细胞肌动蛋白重构，进而促进乳腺癌细胞的迁移和侵袭。本研究有助于阐明乳腺癌转移的分子机制，并为激素依赖性肿瘤提供新的治疗靶点；（5）通过系列研究探索了选择性雌激素受体激动剂（SERM）、植物雌激素（GST）、雌激素代谢中间产物（2-ME）的拟雌激素效应，为临床寻找低副作用、高生理效能雌激素替代药物提供选择。

近年来结合现代治疗学前沿研究，开展了生物反馈疗法的研究，建立国内第一个研究生物反馈疗法生理机制的实验室，主要学术成就包括：（1）率先将应用生物反馈疗法（包括HRV-Biofeedback、EMG-Biofeedback）对高血压前期实施干预，并发现心理差异与高血压前期的成因的关系及对生物反馈疗效存的影响；（2）通过引入HRV的时域、频域和非线性分析参数，发现了生物反馈中受试者的HRV增加，lnLF／lnHF降低，IBI的近似熵（ApEn）值下降，揭示生物反馈过程中自主神经系统活动的变化规律；（3）生物反馈的中枢神经机制研究：采用了PET-CT、脑电近似熵、脑电功率谱等现代检测和分析技术研究生物反馈的中枢神经机制，发现了生物反馈状态中脑电ApEn、心电ApEn和脑电－心电间Cross-ApEn增高，且脑部变化以额叶为主；揭示了包括右侧楔前叶、左侧岛叶、右侧前扣带回等脑区在生物反馈调节中的变化现象及机制。

刘先国，男，教授，博士生导师。

中山医学院副院长、生理学教研室主任、广东省生理学会理事长、广东省疼痛医学会常委，中国生理学会理事及神经科学专业委员会委员，《生理学报》编委会委员。1982年毕业于延边医学院医疗系，获学士学位；1988年获白求恩医科大学硕士学位；1992—1999年先后在德国海德堡大学生理研究所和德国基尔大学研究所攻读博士学位和做博士后。1995年5月被原中山医科大学作为学科带头人引进回国，任教授、博士生导师。

刘先国教授课题组为中山大学疼痛研究中心，其任中心主任。主要成员有：信文君副教授、那晓东副教授、周利君副教授、臧颖副教授、庞瑞平讲师和魏绪红讲师。现有如下研究方向：病理性疼痛的中枢和外周机制；记忆障碍的机制；焦虑和忧郁症的机制；药物成瘾的机制和化疗药物副作用的机制。现已在SCI收录的期刊上发表了四十余篇研究论文，获得

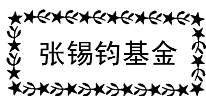
20余项基金资助，其中国家自然科学基金10项。主要学术成就包括：（1）首次报道了强直电刺激和损伤外周神经可引起脊髓背角C纤维诱发电位的长时程增强（LTP），并对其细胞和分子机制进行了系统的研究。目前脊髓背角LTP被认为是痛觉过敏的突触模型，被世界上十几个实验室采用。在今年召开的第十三届世界疼痛大会（蒙特利尔）开设了专题讨论会，专门讨论相关进展，我们应邀做了报告；（2）我们发现神经损伤引起中枢和外周神经系统TNF－α及其受体1（TNFR1）显著上调。过表达的TNF-α可通过上调DRG神经元的TTX-敏感（Nav1.3）和TTX-不敏感（Nav1.8和Nav1.9），导致病理性疼痛，通过破坏海马突触的结构和功能，引起工作记忆障碍；（3）我们发现抗肿瘤药物肽素通过上调背根神经神经元的趋化因子表达，导致单核细胞浸润，引起末梢神经病。

冯鉴强，男，博士，教授。

中山大学中山医学院生理学教授、博士生导师、医学博士。1973年毕业于原中山医科大学（前中山医学院）临床医学专业，并留校在生理学教研室任教。在工作期间，先后获得医学硕士学位和医学博士学位，并于1991年被国务院学位办公室及原国家教委联合授予“做出突出贡献的中国博士学位获得者” 称号。在1993年至1995年获得“何-何-李”奖学金到美国斯坦福大学医学院麻醉科攻读博士后，并被聘为“research fellow”，从事疼痛原理及镇痛机制研究。长期从事生理学教学工作，成效显著。培养博士研究生19人，硕士研究生13人。获得广东省优秀教学成果奖一等奖2项，教学管理优秀成果奖二等奖2项。获得广东省教学科研基金3项。在科研工作方面，先后获得国家自然科学基金、卫生部科研基金及广东省自然科学基金等9项，获得卫生部科技进步奖3等奖1项，广东省科技进步奖3等奖3项，广东省医学科技进步奖2等奖2项，3等奖1项。在国内核心期刊（《生理学报》、《中国应用生理学》、《中国药理学通报》、《中国病理生理学》等）及国外著名杂志（Pain、British Journal of Anesthesia、Brain Research、Apoptosis、Bain Behavior and Immunology、Life Sciences、Neuroscience Letter 和 Plos One 等）发表科研论文80多篇。在1996年至2002年曾先后担任中山医科大学校长助理和教务处长及中山大学中山医学院副院长。现为《生理学》（第二版，科学出版社，2011）的主编。

蒋斌，男，博士生导师。

1998年于中国科学技术大学获得博士学位，师从视觉神经生物学寿天德教授。1998年12月—2003年8月在日本大阪大学医学院做博士后研究，师从日本神经科学学会会长津本忠治（Tadaharu Tsumoto）教授。2003年9月—2005年9月在美国约翰霍普金斯大学医学院继续博士后研究，师从Alfredo Kirkwood 教授。2005年10月起任日本国理化学研究所、脑科学综合研究中心“大脑皮层回路可塑性”核心研究组研究员。2009年底作为中山大学“百人计划”引进人才回国组建神经生物学独立实验室，受聘为中山医学院生理学教授。研究工作主要集中在分子和细胞水平上探讨视觉皮层功能发育可塑性发生的生理机制。



编者按：2013 年 10 月 12-13 日中国生理学会张锡钧基金会第十二届全国青年优秀生理学学术论文交流会在湖南长沙顺利召开。由各省生理学会推荐的 37 名参赛选手的论文参加评选，会议展示了选手们近 3 年来在生理学研究方面所取得的最新研究成果。经过专家对参评者论文和现场报告的综合评判，评出一等奖、二等奖、三等奖、特别奖、最佳表达奖、最佳答辩奖和最佳图表奖共 11 名。从 2013 年第 6 期开始，《生理通讯》将陆续转载获奖者的参评论文各一篇，以飨读者。

The Zinc Finger Protein ZBTB20 Regulates Transcription of *Fructose-1,6-Bisphosphatase* 1 and β-Cell Function in Mice

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Abstract

Background & Aims: Fructose-1,6-bisphosphatase (FBP)-1 is a gluconeogenic enzyme that regulates glucose metabolism and insulin secretion in β−cells, but little is known about how its transcription is controlled. The zinc finger protein ZBTB20 regulates glucose homeostasis, so we investigated its effects on expression of FBP-1. ***Methods:*** We analyzed gene expression using real-time reverse−transcription polymerase chain reaction, immunoblotting, and immunohistochemistry. We generated mice with β-cell–specific disruption of *Zbtb20* using Cre/LoxP technology. Expression of Zbtb20 in β-cells was reduced using small interfering RNAs, and promoter occupancy and transcriptional regulation were analyzed by chromatin immunoprecipitation and reporter assays. ***Results:*** ZBTB20 was expressed at high levels by β-cells and other endocrine cells in islets of normal

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Abbreviations: ChIP, chromatin immunoprecipitation; FBPase, fructose-1,6-bisphosphatase; Fru-6-P, fructose-6-phosphate; Fru-1,6-P2, fructose-1,6-bisphosphate; Fru-2,6-P2, fructose-2, 6-bisphosphate; G-6-P, glucose-6-phosphate; GK, glucokinase; GSIS, glucose-stimulated insulin secretion; HFD, high fat diet; HIF1, hypoxia-inducible factor 1; PFK, phosphofructokinase.**Disclosure:** All authors have no potential financial conflicts about this work.

Author Contributions: W.Z. and M.G. contributed to study concept and design, Y.Z., Z.X., L.Z., L.L., H.Z., G.Z., W.Z., and X.M. contributed to data acquisition, P.H. contributed to material support, Y.Z., Z.X., Z.L, and W.Z. contributed to data analysis and interpretation, W.Z. and Y.Z. wrote the paper, and W.Z. contributed to study supervision.

mice; expression levels were reduced in islets from diabetic *db/db* mice. Mice with β-cell–specific knockout of Zbtb20 had normal development of β-cells, but had hyperglycemia, hypoinsulinemia, glucose intolerance, and impaired glucose-stimulated insulin secretion. Islets isolated from these mice had impaired glucose metabolism, adenosine triphosphate production, and insulin secretion after glucose stimulation in vitro, although insulin secretion returned to normal levels in the presence of KCl. ZBTB20 knockdown with small interfering RNAs impaired glucose-stimulated insulin secretion in the β-cell line MIN6. Expression of Fbp1 was up-regulated in β-cells with ZBTB20 knockout or knockdown; impairments to glucose-stimulated insulin secretion were restored by inhibition of FBPase activity. ZBTB20 was recruited to the *Fbp1* promoter and repressed its transcription in β-cells. ***Conclusions:*** The transcription factor ZBTB20 regulates β-cell function and glucose homeostasis in mice. It might be a therapeutic target for type 2 diabetes mellitus.
Keywords: pancreas; mouse model; RIP-Cre; repressor.

Introduction

Pancreatic β-cells play a critical role in glucose homeostasis by secreting insulin in a tightly controlled manner, the progressive dysfunction of which is one of the pathological characteristics of type 2 diabetes mellitus (T2DM) [1]. Through a metabolism-secretion coupling system, β-cells can precisely sense glucose stimulation by its conversion into metabolic intermediates including adenosine triphosphate (ATP) and accordingly adjust insulin secretion by closure of ATP-sensitive K^+ channels and influx of Ca^{2+} [2-3]. In past decades, some critical components of glucose-sensing machinery in β-cells have been established, which include the facilitative glucose transporter GLUT2, glucokinase (Gk), and the glycolytic enzyme phosphofructokinase (PFK) [1, 4, 5]. Recent reports indicate that fructose-1,6-bisphosphatase (FBPase), a rate-limiting gluconeogenic enzyme, plays a role in regulating glucose sensing and insulin secretion of β-cells [6-8].

FBPase controls the futile recycling between fructose-6-phosphate and fructose-1,6-bisphosphate by coupling with PFK, thus regulating the glycolytic/gluconeogenic flux [9]. FBPase has FBP1 and FBP2, 2 distinct isoenzymes in mammals that are preferentially expressed in liver and muscle, respectively [10]. FBP1 is also expressed in human and rodent islets [6] and acts as a regulator of glucose metabolism and insulin secretion in β-cells [8]. More importantly, it is implicated in β-cell dysfunction in the pathogenesis of T2DM. *Fbp1* expression is up-regulated both in the islets from patients with T2DM and in the β-cells after exposure to hyperglycemia or treatment with lipid [7, 11-13]. Transgenic overexpression of *Fbp1* in β-cells leads to impaired glucose-stimulated insulin secretion (GSIS) [7]. However, little is known about the mechanisms underlying the transcriptional regulation of FBP1.

Zinc finger and BTB domain-containing protein 20 (ZBTB20, also known as DPZF, HOF, and ZFP288) belongs to a subfamily of zinc finger proteins containing C2H2 Krüppel-type zinc fingers and BTB/POZ domains[14-15]. Due to alternative translational initiation, ZBTB20 protein has 2 isoforms, both containing these 2 domains[15]. ZBTB20 plays important roles in multiple systems, as suggested by the severe phenotypes in the mice lacking ZBTB20, which mainly include growth retardation, premature death, hypoglycemia, and hippocampal defect [16-17]. The hypoinsulinemic hypoglycemia phenotype is most likely caused by certain unknown defects beyond a feeding problem, because considerable amounts of milk are consistently present in the stomachs of *Zbtb20* knockout mice, suggesting that ZBTB20 may be an important player in glucose homeostasis [16]. Given the critical role of pancreatic exocrine and endocrine cells in glucose metabolism, we

analyzed expression pattern and biological functions of ZBTB20 in the pancreas. In this study, we show that ZBTB20 protein is abundantly expressed by pancreatic islet β-cellsand plays an important role in regulating β-cell function through transcriptional repression of *Fbp1*.

Results

Expression of ZBTB20 in Pancreatic Endocrine Cells

To investigate the potential role of pancreatic ZBTB20 in glucose homeostasis, we first characterized its expression pattern in mouse pancreas. Immunohistochemical staining revealed that ZBTB20 protein was highly expressed by pancreatic islets and ductal cells of adult mice but absent in exocrine acini, while there was no positive staining detected in the pancreas from ZBTB20 global knockout mice (Figure 1*A*). In the islets, ZBTB20 protein was shown by double immunofluorescence staining to be abundantly expressed in insulin-producing β-cells, as well as in glucagon-producing α-cells, somatostatin-producing δ-cells, and pancreatic polypeptide-producing pancreatic polypeptide cells (Figure 1*B*). Both isoforms of ZBTB20 were detected in mouse islets, with the short one preferentially expressed. However, in mouse β-cell lines MIN6 and β-TC, these 2 isoforms were comparably expressed at high levels (Figure 1*C*). Under pathophysiological conditions, *Zbtb20* messenger RNA (mRNA) expression was markedly decreased in the islets either from diabetic db/db mice compared with their control littermates (Figure 1*D*) or from the mice fed a high-fat diet compared with those fed normal chow (Figure 1*E*). These findings implied that ZBTB20 in islet endocrine cells, especially in β-cells, might have a role in regulating glucose homeostasis and in the pathogenesis of diabetes.

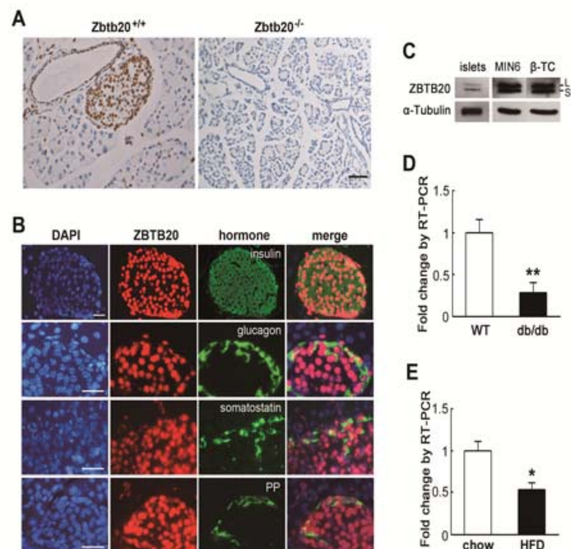


Figure 1. Expression of ZBTB20 in mouse pancreatic islets.
(A) Pancreatic sections from adult wild-type or ZBTB20 global knockout mice were examined by immunohistochemical staining with anti-Zbtb20 antibody 9A10, and developed by 3,3'-diaminobenzidine tetrahydrochloride. *Scale bar = 50 um.* (B) Pancreatic islets were analyzed by double immunofluorescence using anti-ZBTB20 antibody 9A10 and antibodies against insulin, glucagon, somatostatin, or pancreatic polypeptide, respectively. The nuclei were counterstained by 4',6-diamidino-2-phenylindole. *Scale bar = 25 um.* (C) Western blot analysis for ZBTB20 protein expression in mouse islets and β-cell lines MIN6 and β-TC. S, short isoform; L, long isoform. (D,E) Quantitative RT-PCR analysis for Zbtb20 mRNA expression in the islets from db/db and control mice at the age of 2 months (D) or from adult C57BL/6 mice fed a high-fat diet (HFD) or normal chow for 4 months (E). *, P<.05 vs normal chow, **, P<.01 vs WT.

Ablation of ZBTB20 in β-Cells Results in Glucose Intolerance and Impaired Insulin Secretion

To determine the role of ZBTB20 in islet β-cells in vivo, we used the Cre/LoxP system to generate β-cell-specific *Zbtb20* knockout mice (hereafter referred to asβ-ZB20KO). Mice with a floxed *Zbtb20* gene [18] were crossed with the transgenic Cre mice under the control of rat insulin promoter (hereafter referred to RIP-Cre), a line that was reported to mediate loxP recombination specifically in islet β-cells and

exhibit normal glucose tolerance [19-21]. PCR analysis of genomic DNA showed that *Zbtb20* exon 6 was efficiently disrupted in β-ZB20KO islets (Figures 2*A* and *B*), and the residual signal of exon 6 reflects the unrecombined *Zbtb20^{flox}* allele, most likely from non-β-cells in the islets. Efficient deletion of the *Zbtb20* gene in β-ZB20KO islets was confirmed at the mRNA level by real-time RT-PCR and at the protein level by immunoblot analysis with anti-ZBTB20 antibody (Figures 2*C* and *D*). Immunohistochemical analysis of β-ZB20KO islets showed that ZBTB20 protein was largely disrupted in the β-cells but still present in the non-β-cells (Figure 2*E*). To evaluate the tissue specificity of RIP-Cre-mediated *Zbtb20* deletion, we also examined ZBTB20 expression in other tissues outside the pancreas. We found that ZBTB20 protein expression was not significantly affected in hypothalamus, liver, or skeletal muscle from β-ZB20KO mice compared with their control counterparts (Figure 2*D*), which is consistent with previous reports about this RIP-Cre line [19, 21].

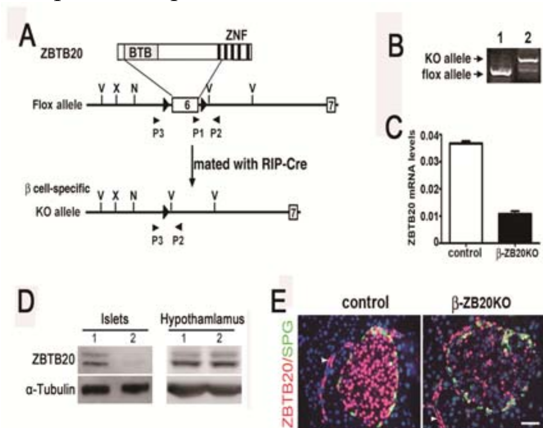


Figure 2. Generation of β-ZB20KO mice.

(A) Schematic demonstration of the deletion of exon 6 from Zbtb20 gene in β-cells Cre recombination. Primers P1 and P2 were used for PCR analysis for exon 6 and its downstream loxP site, whereas P2 and P3 were used to amplify the recombined allele. (B) PCR analysis for Cre-mediated Zbtb20 deletion in islets. Genomic DNA from Zbtb20$^{flox/flox}$ (lane 1) or β-ZB20KO (lane 2) islets was subjected to PCR analysis with primers P1, P2, and P3. (C) Quantitative RT-PCR analysis for Zbtb20 mRNA in β-ZB20KO islets. (D) Western blot analysis for ZBTB20 expression in islet and hypothalamus from Zbtb20$^{flox/flox}$ (lane 1) or β-ZB20KO (lane 2) mice. (E) Efficient and specific deletion of Zbtb20 in β-cells shown by double immunofluorescence staining of mouse anti-ZTBT20 antibody 9A10 (red) and mixed rabbit antibodies against somatostatin, pancreatic polypeptide, and glucagon (SPG, green). White arrowhead indicates ZBTB20-positive ductal cells. *Scale bar = 50 um*.

Born at the expected frequencies, β-ZB20KO mice were fertile, grew normally, and did not differ in size or weight from their littermate controls. In addition, there was no significant difference of food intake, oxygen consumption, CO2 production, or activity between β-ZB20KO and control adult mice, indicating that ZBTB20 ablation mediated by this RIP-Cre line did not affect energy homeostasis.

To assess the effect of the β-cell *Zbtb20* ablation on glucose homeostasis, we measured plasma glucose and insulin levels in fasted and random-fed states. At the age of 2 months, no significant difference in fasting blood glucose level was observed in either male or female β-ZB20KO mice compared with *Zbtb20$^{flox/flox}$* (hereafter referred as F/F) or heterozygous *Zbtb20$^{flox/+}$*; *RIP-Cre* (hereafter referred as F/+/Cre) control littermates (Figure 3*A*). However, starting from 4 months of age, both male and female β-ZB20KO mice showed progressive fasting hyperglycemia (Figure 3*A*), and their fasting insulin levels were much lower than in control mice (Figure 3*B*). Then we performed the glucose tolerance test on overnight-fasted adult mice. After intraperitoneal injection of glucose at half of the regular dose, blood glucose level peaked at approximately 200 mg/dL at 15 minutes in control mice. In contrast, glucose tolerance was severely impaired in β-ZB20KO mice, with a peak blood glucose level of approximately 450 mg/dL at 30-60 minutes after glucose injection (Figure 3*C*). Moreover, β-ZB20KO mice showed normal

insulin tolerance (Figure 3*D*), indicating their glucose intolerance was most likely to result from β-cell dysfunction.

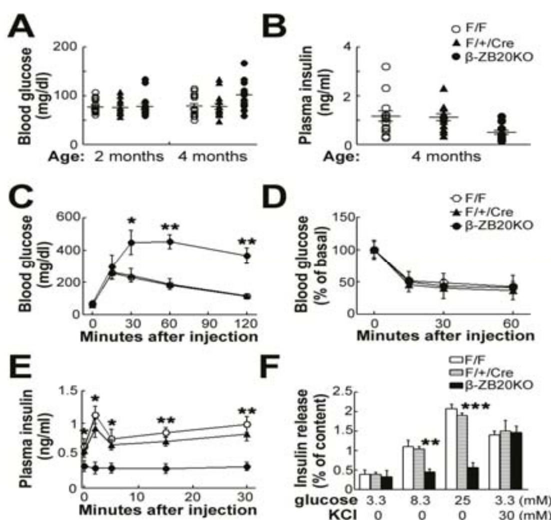


Figure 3. Impaired glucose tolerance and insulin secretion in β-ZB20KO mice.
(A) Blood glucose levels of overnight fasted mice at the ages of 2 and 4 months (n=14~15). (B) Plasma insulin levels of overnight fasted mice at the age of 4 months (n=18). (C) Intraperitoneal glucose tolerance test results showed glucose intolerance in β-ZB20KO mice at the age of 2—3 months (n=9). (D) Insulin tolerance test result showed normal insulin tolerance in β-ZB20KO mice (n=8). (E) In vivo insulin secretion test results after glucose challenge (n=8). (F) In vitro insulin secretion of islets after glucose stimulation (n=4). The isolated islets were stimulated with glucose at the presence or absence of KCl for 1 hour. The insulin levels in culture supernatants were normalized by total cellular insulin contents. *, P<.05; **, P<.01; ***, P<.001 vs control.

Then we measured plasma insulin levels after glucose stimulation, and found that β-ZB20KO mice displayed a loss of first-phase and delayed second-phase insulin secretion (Figure 3*E*). It is noteworthy that some RIP-Cre lines themselves show impaired glucose tolerance [22]. Therefore, in these glucose tolerance and insulin secretion experiments, we included both RIP-Cre and heterozygous F/+/Cre mice as control, neither of which showed a significant difference compared with wild-type or *Zbtb20*$^{flox/flox}$ control (Figure 3*C*, 3*E*, and data not shown). These were consistent with previous reports that this RIP-Cre line has normal glucose tolerance [19, 21]. Collectively, these results suggested that the β-cell dysfunction in β-ZB20KO micewas caused by β-cell-specific deletion of ZBTB20.

Defects in Glucose Sensing and Insulin Secretion of β-ZB20KO Islets

To determine whether the β-cell dysfunction in β-ZB20KO micewas associated with morphological abnormality, we performed histologic examination. By immunostaining of glucagon, somatostatin, and pancreatic polypeptide produced by non-β-cells in islets, either islet architecture or β-cell mass did not differ between control and β-ZB20KO mice. In addition, their pancreatic insulin content from the acid-ethanol extracts did not show a significant difference.

We next evaluated glucose-induced insulin secretion from isolated islets. Upon the basal level stimulation of 3.3 mmol/L glucose, β-ZB20KO and control islets secreted a comparable amount of insulin (Figure 3*F*). However, when stimulated with high levels of glucose, β-ZB20KO islets displayed impaired insulin secretion compared with their control counterpart. Interestingly, the insulin secretion defect was glucose specific because insulin secretion was normal in response to 30 mmol/L KCl, which mediates membrane depolarization and triggers insulin secretion. This suggested that the insulin secretion pathway downstream from the ATP-sensitive K^+ channel may be normal in ZBTB20-deficient β-cells, and their defect of GSIS could occur at the glucose metabolic pathway. To confirm this hypothesis, we isolated islets and measured their glucose utilization and the ratios of cellular ATP to ADP *in vitro*. Upon the stimulation of 3.3 mmol/L glucose, there was no significant difference of either glucose utilization or the ATP/ADP ratios between β-ZB20KO and control islets. However, when stimulated with high levels of glucose, β-ZB20KO islets showed greatly

impaired glucose utilization and reduced ATP/ADP ratios compared with their control counterparts (Figures 4*A* and *B*). The decrease in ATP/ADP ratio is of sufficient magnitude to cause the impairment of GSIS.

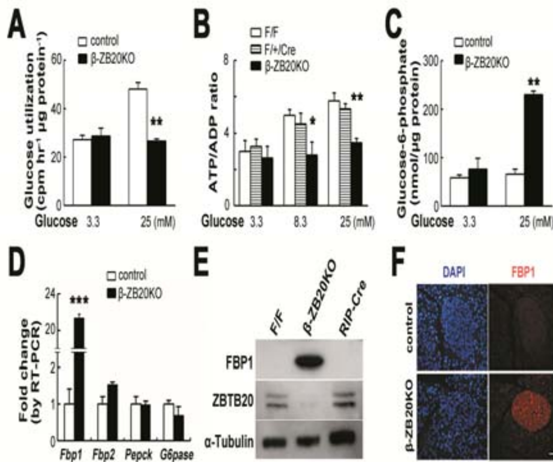


Figure 4. Impaired glucose metabolism and dysregulated expression of Fbp1 in β-ZB20KO islets. (A) Glucose utilization of isolated islets. (B) Cellular ATP/ADP ratios of isolated islets after glucose stimulation. (C) Elevated cellular contents of G-6-P in β-ZB20KO islets. (D) mRNA levels of gluconeogenic genes were determined in islets by quantitative RT-PCR. (E and F) Increased FBP1 protein expression in β-ZB20KO islets shown by (E) Western blotting and (F) immunofluorescence staining. Scale bar = 50 um. *, P<.05, **, p<.01 ***, P<.001 vs control. N=4 per group.

To further assess glucose metabolism, we measured cellular contents of G-6-P in islets. Under basal condition, there was no significant difference of G-6-P contents between control and β-ZB20KO islets. To our surprise, upon stimulation with high glucose levels, β-ZB20KO islets had markedly increased contents of G-6-P compared with control islets (Figure 4C), which excluded the possibility of decreased uptake of glucose by β-cells and/or impaired glucose phosphorylation by glucokinase. These findings indicate that ZBTB20-deficient β-cells had a significant defect of glucose metabolism, most likely occurring downstream of G-6-P. We then tested nonglucose fuels entering glycolysis distal to G-6-P. α-Ketoisocaproate is transaminated with glutamate to leucine and α-ketoglutarate in β-cells, which, in turn, are metabolized to produce intracellular signals such as ATP for insulin secretion [23]. After stimulation with 20 mmol/L α-ketoisocaproate in the presence of low glucose levels, β-ZB20KO islets released comparable amounts of insulin relative to control islets, suggesting that glycolysis distal to G-6-P could be normal in ZBTB20-deficient β-cells. Taken together, we reasoned that the defects of glycolysis might occur not far from downstream G-6-P in ZBTB20-deficient β-cells, which at least partly contributed to the impairment of glucose sensing and insulin secretion.

Altered Gene Expression in β-ZB20KO Islets

To determine the molecular basis for the insulin secretion defects in response to glucose, we performed quantitative real-time RT-PCR analysis on the isolated islets. β-ZB20KO and control islets exhibited comparable mRNA expression levels of insulin-encoding gene *Ins2*, glucagon-encoding gene *Gcg*, somatostatin-encoding gene *Sst*, pancreatic polypeptide-encoding gene *Ppy*, peroxisome proliferator-activated receptor γ coactivator 1α-encoding gene*Ppargc1α,* and MODY genes *Pdx1, Hnf1α, Hnf1β*, *Hnf4α*, *and Gk*, whereas *Neuro D1* was mildly increased in β-ZB20KO islets. Of the glucose transporters *Glut1*, *Glut2*, and *Glut3* detected, *Glut1* mRNA expression was markedly reduced in β-ZB20KO islets relative to control islets, whereas *Glut2* showed a trend toward increased expression. In addition to *Gk*, other glycolytic genes including *G6pi*, *Pfk*, *Pgm,* and *L-PK* did not display significant changes of the mRNA expression levels in β-ZB20KO islets. Both *Pepck* and *G6Pase*, the key gluconeogenic genes, were detected at comparably low levels in β-ZB20KO and control islets, but *Fbp1* mRNA was increased nearly 20-fold in β-ZB20KO islets, whereas *Fbp2* mRNA expression was not significantly altered (Figure 4*D*). Then we confirmed FBP1 expression by Western blot and

immunohistochemical analyses. Consistent with our previous report [8], FBP1 protein was undetectable by Western blotting or immunostaining by anti-FBP1 antibodies in the normal islets from either *ZBTB20^flox/flox^* or *RIP-Cre* mice. However, it was specifically detected at the high levels from the lysates of β-ZB20KO islets (Figure 4*E*). Immunostaining also revealed abundant expression of FBP1 protein in islet β-cells from β-ZB20KO mice (Figure 4*F*). This is consistent with the increase of cellular G-6-P at its upstream. Given the role of FBP1 in the regulation of GSIS of β-cells [7-8], we reasoned that up-regulated expression of FBP1 at least partlycould lead to the impairment of glucose sensing and insulin secretion of ZBTB20-deficient β-cells.

Impaired Insulin Secretion and Increased Fbp1 Expression in Zbtb20 Knockdown β-Cells

We next investigated the role of ZBTB20 in a glucose-responsive mouse β-cell model. MIN6 cells were treated with ZBTB20-specific or scrambled control siRNA. As shown in Figure 5*A*, treatment of ZBTB20-specific siRNA led to a significant decrease of ZBTB20 protein in MIN6 cells, whereas scrambled siRNA had no effect on ZBTB20 protein levels. Seventy-two hours after siRNA transfection, MIN6 cells were stimulated with glucose to test their insulin secretion. Compared with scrambled siRNA-transfected control, ZBTB20 knockdown MIN6 cells had comparable cellular insulin contents and basal insulin secretion at 1.1 mmol/L glucose but secreted much lower levels of insulin at 11 mmol/L glucose (Figure 5*B*). Moreover, increased expression of *Fbp1* mRNA was detected by RT-PCR in *Zbtb20* knockdownMIN6 cells or islets (Figures 5*C* and *D*). These results were consistent with that of β-ZB20KO islets, supporting the conclusion that ZBTB20 regulates *Fbp1* expression and insulin secretion in β-cells.

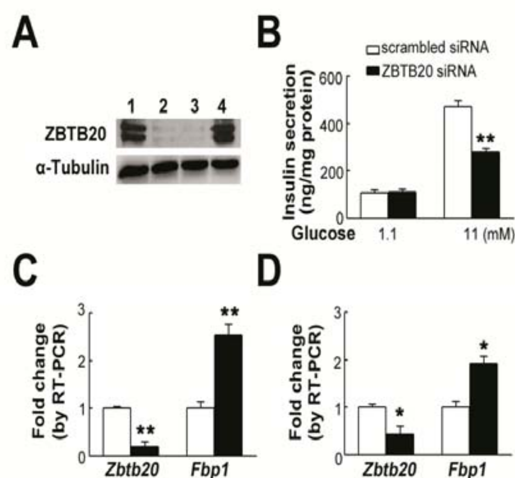


Figure 5. Impaired insulin secretion and increased Fbp1 expression in ZBTB20 knockdown β-cells.
(A) Western blot analysis for ZBTB20 expression in MIN6 cells after treatment for 72 hours with vehicle control (lane 1), Zbtb20 siRNA (lanes 2, 3), or scrambled control siRNA (lane 4). (B) Impaired insulin secretion in Zbtb20 siRNA-treated MIN6 cells. MIN6 cells were treated with siRNA for 72 hours before glucose stimulation for 1 hour. The insulin secretion was normalized by total cellular protein contents. (C and D) Quantitative RT-PCR analysis for Fbp1 expression in siRNA-treated (C) MIN6 cells and (D) islets. *P<.05, **P<.01 vs control. N=4 per group.

Restored Insulin Secretion by Inhibition of FBPase Activity

To determine whether FBP1 is a major mediator in the impaired GSIS of ZBTB20-deficient β-cells, we took a pharmacologic approach to block FBP1 activity in the islets during glucose stimulation. Consistent with our previous observation [8], treatment with 250 μmol/L MB05032, a specific inhibitor of FBPase [24], markedly enhanced GSIS from control islets at 3.3 or 25 mmol/L glucose. β-ZB20KO islets treated with 250 μmol/L MB05032 showed an increasing tendency of insulin secretion at 3.3 mmol/L glucose, but it did not reach statistical significance. Upon stimulation with 25 mmol/L glucose, 250 μmol/L MB05032-treated β-ZB20KO islets secreted significantly higher

levels of insulin compared with the vehicle mock control, however, which was much lower than that of control islets treated with 250 μmol/L MB05032 (Figure 6*A* and *B*). Considering the robust expression of FBP1 protein in β-ZB20KO islets, we reasoned that a higher dose of MB05032 might be required to block its activity in β-cells. Therefore, we treated β-ZB20KO and control islets with 500 μmol/L MB05032. The insulin secretion levels of control islets at 3.3 and 25 mmol/L glucose in the presence of 500 μmol/L MB05032 were further mildly increased compared with 250 μmol/L MB05032. Importantly, β-ZB20KO and control islets treated with 500 μmol/L MB05032 secreted comparable levels of insulin at basal and stimulatory doses of glucose, suggesting that blockade of the increased FBP1 activity in ZBTB20-deficient β-cells could restore their glucose-stimulated insulin secretion. Consistently, treatment of 500 μmol/L MB05032 also led to significant restoration of glucose utilization and ATP/ADP ratios in β-ZB20KO islets at 25 mmol/L glucose (Figure 6*C* and *D*). These findings suggested that up-regulated FBP1 protein in ZBTB20-deficient β-cells might mainly contribute to the impairment of glucose sensing and insulin secretion.

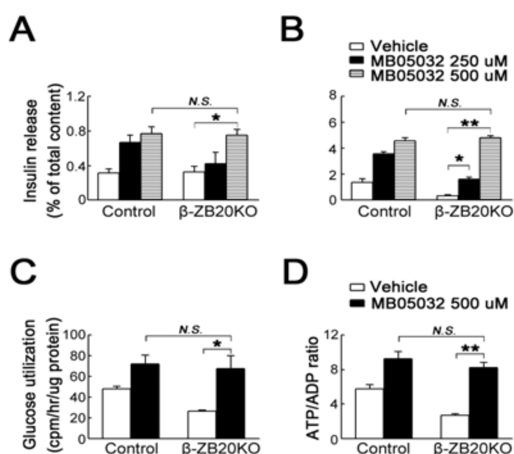


Figure 6. Restoration of glucose metabolism and insulin secretion from β-ZB20KO islets by inhibition of FBP1 activity.
(A and B) Restored insulin secretion from β-ZB20KO islets by FBPase inhibitor MB05032. β-ZB20KO and control islets were stimulated with (A) 3.3 mmol/L or (B) 25 mmol/L glucose for 1 hour in the absence or presence of MB05032 at indicated concentrations, and their insulin secretion was normalized with total cellular insulin content. (C and D) MB05032 treatment improved (C) glucose utilization and (D) cellular ATP/ADP ratios in β-ZB20KO islets at 25 mmol/L glucose. *P<.05; **P<.01 vs vehicle control; N.S., not significant vs control. n = 4 per group.

ZBTB20 Regulates Fbp1 Gene Transcription in β-Cells

To biochemically determine the direct evidence about the regulation of *Fbp1* gene transcription by ZBTB20, we first performed ChIP assay to examine the physiological occupancy of ZBTB20 on the *Fbp1* promoter. In MIN6 cells, ChIP assays were performed with antibodies against ZBTB20, acetylated histone 3 (AcH3, positive control), or control immunoglobulin G as negative control of chromatin recovery, and the recovered chromatin DNA was subjected to PCR analysis using 3 different primer sets for *Fbp1* gene and its upstream loci. As shown in Figure 7, ZBTB20 was recruited on the *Fbp1* promoter spanning about 1 kilobase upstream the transcriptional start site, but not in the unrelated region located 6 kilobases distal to the *Fbp1* gene in MIN6 cells. We further performed ChIP assay on pooled mouse islets and found consistently that ZBTB20 occupied on the *Fbp1* promoter in pancreatic islets.

We next cloned the regulatory region spanning -1653 to +43 of the *Fbp1* gene and constructed luciferase reporter to test whether ZBTB20 might be capable of repressing *Fbp1* promoter activity. Transient overexpression of ZBTB20 in MIN6 cells failed to affect the activity of *Fbp1* promoter-driven luciferase reporter (Figure 7*D*), probably due to their abundant expression of endogenous ZBTB20 protein. On the other hand, ZBTB20 knockdown in MIN6 cells by siRNA treatment resulted in a significant increase of the *Fbp1* promoter-driven reporter activity (Figure 7*E*), indicating that ZBTB20 could repress the transcriptional activity of the *Fbp1* promoter. Combined with the data of increased expression of

Fbp1 both in β-ZB20KO islets and in ZBTB20 knockdown MIN6 cells, these data suggested that ZBTB20 act as a transcriptional repressor of the *Fbp1* gene in β-cells.

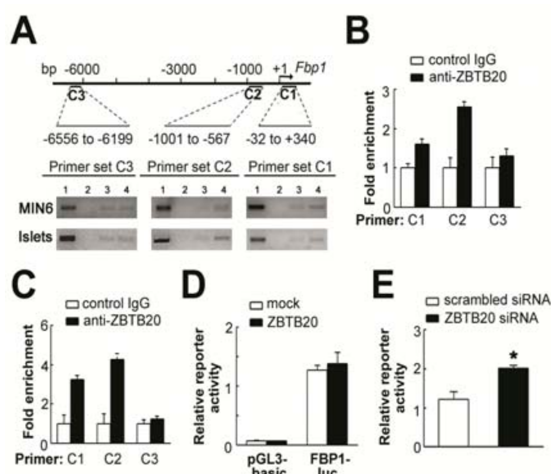


Figure 7. Transcriptional regulation of *Fbp1* by ZBTB20 in β-cells.
(A) The recruitment of ZBTB20 on the *Fbp1* promoter in MIN6 cells and islets. ChIP assays were performed in MIN6 cells and islets using indicated antibodies and primer pairs. Input (lane 1) and no template control (lane 2), control immunoglobulin G (lane 3), and ZBTB20 antibody (lane 4). (B and C) Quantitative PCR-based ChIP analysis for the enrichment of ZBTB20 on the *Fbp1* promoter in (B) MIN6 and (C) islets. All values were normalized by control immunoglobulin G precipitations. Each bar represents the average result from 2 independent ChIP experiments and 4 real-time PCR analyses. (D) Overexpression of ZBTB20 did not significantly affect the *Fbp1* promoter activity in MIN6 cells. (E) Enhanced transcriptional activity of the *Fbp1* promoter-driven reporter by ZBTB20 knockdown in MIN6 cells. MIN6 cells were treated with ZBTB20 siRNA or control siRNA for 4 days before transfection with the *Fbp1* reporter constructs. **P*<.05 vs scrambled siRNA control. N=4 per group.

ZBTB20 Regulates FBP1 Gene Transcription in Hepatocytes

Considering that both *Zbtb20* and *Fbp1* are also expressed in hepatocytes, we further assessed whether ZBTB20 regulated *Fbp1* gene transcription in the liver. To this end, we first examined whether ZBTB20 disruption in hepatocytes led to dysregulated *Fbp1* expression and found that *Fbp1* mRNA levels were increased 40% in the livers from hepatocyte-specific *Zbtb20* knockout mice compared with their littermate control. Moreover, transient overexpression of ZBTB20 in primary ZBTB20-null hepatocytes resulted in dramatic inhibition of *Fbp1* promoter-driven transcription activity. ChIP assay revealed that ZBTB20 was robustly recruited to the *Fbp1* promoter in the liver. Taken together, these data suggested that ZBTB20 could repress *Fbp1* gene transcription both in β-cells and in hepatocytes.

Discussion

Regulation of GSIS by ZBTB20

In the present study, we examined the role of ZBTB20 in β-cell biology and function. Our findings provide compelling evidence that β-cell ZBTB20 regulates glucose sensing and insulin secretion via transcriptional repression of FBP1. First, *Zbtb20* gene is abundantly expressed in islet β-cells. Second, β-cell-specific ablation of *Zbtb20* results in decreased glucose utilization and ATP production, as well as impaired insulin secretion. Similar results can be obtained in β-cell lines by RNA interference. The β-cell defect is relatively glucose specific, because the insulin secretion was normal in response to KCl stimulation. Third, *Fbp1* gene expression is up-regulated both in ZBTB20-deficient β-cells and in *Zbtb20* siRNA-treated β-cells or islets. FBP1 acts as a regulator of GSIS in β-cells, and its up-regulation results in the impairment of GSIS [7-8]. Blockage of FBP1 activity with its specific inhibitor MB05032 completely restored the impaired glucose utilization and insulin secretion of ZBTB20-deficient islets in vitro. This provides additional evidence to support inhibition of FBP1 as a therapeutic target for the treatment of T2DM. Last, ZBTB20 directly regulates *Fbp1* transcription in β-cells. ZBTB20 binds to *Fbp1* promoter by ChIP assay, and functions as a transcriptional repressor of the *Fbp1* gene. The transcription regulatory mechanism

of ZBTB20 is under further investigation. Taken together, our results for the first time establish the role of ZBTB20 in β-cell function and identify *Fbp1* as one of its target genes in β-cells.

FBP1 regulation in β-cell dysfunction

As a gluconeogenic enzyme, FBP1 is critical for liver endogenous glucose production. FBP1 was recently recognized as an important regulator of glucose metabolism and insulin secretion of β-cells [7-8]. By coupling with PFK and finely tuning the conversion between fructose-1,6-bisphosphate and fructose-6-phosphate, FBPase controls the glycolytic/gluconeogenic flux, decreasing substrate availability for glycolysis. The physiological role of FBP1 in β-cells is neglected due to its low expression. Our previous work found that FBP1 mRNA levels in mouse islets were 20-fold higher than that in muscle, and treatment of mouse islets with FBP1 inhibitor could robustly augment GSIS both *in vitro* and *in vivo*, which argues its physiologic functions in β-cells [8]. Nevertheless, the regulatory mechanisms of *Fbp1* expression in β-cells remain elusive. Our results suggest that low expression of *Fbp1* in normal β-cells is mainly due to the negative regulation by ZBTB20. Thus, we postulate that ZBTB20 functions as a physiologic facilitator of GSIS in β-cells through transcriptional repression of *Fbp1*. This extends our knowledge about the regulatory networks of glucose-sensing machinery and insulin secretion in β-cells and highlights the critical role of regulated *Fbp1* in β-cell function.

In diabetic human and rodent islets, *Fbp1* expression is significantly increased, which can be a contributing factor in the impairment of GSIS [7]. Given the critical role of ZBTB20 in the transcriptional regulation of *Fbp1*, we reason that the dysregulated *Fbp1* expression in β-cells may result from decreased expression and/or compromised repressive activity of ZBTB20, which is partly confirmed by our observation of decreased *Zbtb20* expression in diabetic islets. Subjects with T2DM characteristically have pronounced impairment of first-phase insulin secretion [25-26]. This defect is islet intrinsic and relatively glucose specific in the earlier stages of the disease [27-28]. Some factors (eg,hypoxia-inducible factor 1α and ARNT/hypoxiainducible factor 1β) have been shown to contribute to altered function of diabetic β-cells [29-30]. Our results suggest that ZBTB20 may be another important player in β-cell dysfunction during the pathogenesis of T2DM.

T2DM is a genetically heterogeneous disease, with the most common forms resulting from the complex interplay of many different pathways under the control of genetic and environmental factors [31]. In humans, it is unlikely that a genetic alteration of ZBTB20 itself causes the disease, because the mice lacking ZBTB20 have severe hypoglycemia and high premature mortality [16]. Besides β-cells, *Zbtb20* is also widely expressed in islet non-β-endocrine cells, brain, skeletal muscle, heart, kidney and liver [18], which raises the possibility that its essential role in glucose homeostasis may be mediated by different tissues and mechanisms. In the whole body *Zbtb20* knockout mice, altered β-cell function is probably masked by the phenotype of severe hypoglycemia, with hypoinsulinemia being a probable compensatory response. Therefore, we speculate that the pathogenic significance of ZBTB20 in T2DM is probably associated with its decreased expression or functional abnormality in β-cells, which may result from the combination of acquired and genetic alterations.

In summary, these studies show that β-cell ZBTB20 regulates glucose-stimulated insulin secretion through transcriptional repression of *Fbp1*, which helps to unravel the biochemical basis of β-cell dysfunction in T2DM and to validate *Fbp1* as a potential therapeutic target for the treatment of T2DM.

Materials and Methods

Mouse Models

β-cell-specific ZBTB20 knockout mice were generated by crossing *Zbtb20* flox mice[18] with RIP-Cre transgenic mice[19].

Metabolic Analyses

Glucose tolerance test was performed by intraperitoneal injection of D-glucose (1 g/kg body weight) into overnight-fasted mice, and insulin tolerance test was performed by intraperitoneal injection of human regular insulin (0.75 U/kg body weight; Sigma, St Louis, MO) into randomly fed mice. For *in vivo* insulin release assay, glucose (3 g/kg body weight) was intraperitoneally injected.

Islet Isolation and In Vitro Functional Assays

Islets were isolated from adult mice by the intraductal injection of collagenase P (Roche Diagnostics, Mannheim, Germany) and assessed for insulin secretion, glucose utilization, and ATP/ADP ratios as previously described[8]. For some experiments, islets were stimulated with glucose in the presence of 30 mmol/L KCl or 250~500 μmol/L MB05032. Cellular glucose-6-phosphate (G-6-P) was detected using a G-6-P assay kit (BioVison, Mountain View, CA).

Real-Time Reverse-Transcription Polymerase Chain Reaction

Quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed as previously described using total RNA[18]. PCR reaction contained SYBR green (Invitrogen, Carlsbad, CA) and specific primers for each gene, and every plate included 36B4 gene as internal control. Primer sequences are available on request.

Reporter Assay

The mouse *Fbp1* promoter (-1653 to +43) was cloned into the pGL3-basic vector (Promega, Madison, WI). MIN6 cells and mouse primary hepatocytes were transiently transfected in 24-well plates with plasmids by Effectene (Qiagen, Valencia, CA). Forty-eight hours after transfection, cells were disrupted and subjected to dual luciferase assay with RL-SV40 as internal control.

RNA Interference

MIN6 cells or single cells dispersed from isolated islets were transfected with double-stranded small interfering RNA (siRNA) using siRNA transfection reagent (Dharmacon, Thermo Scientific, Lafayette, CO). Three days later, RNA interference efficiency was determined by Western blotting with anti-ZBTB20 monoclonal antibody 9A10, and insulin secretion was detected by enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). ZBTB20 siRNA sense sequence is GUCAGUAACAGCUCCGAUAUU.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) assays were performed as previously described [18] with some modifications. After crossing and sonication, cell lysates were immunoprecipitated with indicated antibodies. The eluted genomic DNA from immunoprecipitates was subjected to PCR amplification using primers for the different regions of the *Fbp1* promoter.

Statistical Procedures

Unless otherwise indicated, all values are expressed as mean ± SEM. Statistical analyses were performed using Student *t* test or analysis of variance followed by post hoc comparisons, and the null hypothesis was rejected at the 0.05 level.

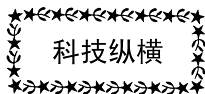
References

1. Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in beta-cell function. Nature 2001;414:788-91.
2. Herman MA, Kahn BB. Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. J Clin Invest 2006;116:1767-75.
3. Schuit FC, Huypens P, Heimberg H, et al. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes 2001;50:1-11.
4. Matschinsky FM, Glaser B, Magnuson MA. Pancreatic beta-cell glucokinase: closing the gap

between theoretical concepts and experimental realities. Diabetes 1998;47:307-15.

5. Ristow M, Carlqvist H, Hebinck J, et al. Deficiency of phosphofructo-1-kinase/muscle subtype in humans is associated with impairment of insulin secretory oscillations. Diabetes 1999;48:1557-61.
6. Yanez AJ, Bertinat R, Spichiger C, et al. Novel expression of liver FBPase in Langerhans islets of human and rat pancreas. J Cell Physiol 2005;205:19-24.
7. Kebede M, Favaloro J, Gunton JE, et al. Fructose-1,6-bisphosphatase overexpression in pancreatic beta-cells results in reduced insulin secretion: a new mechanism for fat-induced impairment of beta-cell function. Diabetes 2008;57:1887-95.
8. Zhang Y, Xie Z, Zhou G, et al. Fructose-1,6-bisphosphatase regulates glucose-stimulated insulin secretion of mouse pancreatic beta-cells. Endocrinology 2010;151:4688-95.
9. Pilkis SJ, Granner DK. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 1992;54:885-909.
10. Tillmann H, Eschrich K. Isolation and characterization of an allelic cDNA for human muscle fructose-1, 6-bisphosphatase. Gene 1998;212:295-304.
11. Zitzer H, Wente W, Brenner MB, et al. Sterol regulatory element-binding protein 1 mediates liver X receptor-beta-induced increases in insulin secretion and insulin messenger ribonucleic acid levels. Endocrinology 2006;147:3898-905.
12. Xiao J, Gregersen S, Kruhoffer M, et al. The effect of chronic exposure to fatty acids on gene expression in clonal insulin-producing cells: studies using high density oligonucleotide microarray. Endocrinology 2001;142:4777-84.
13. Laybutt DR, Sharma A, Sgroi DC, et al. Genetic regulation of metabolic pathways in beta-cells disrupted by hyperglycemia. J Biol Chem 2002;277:10912-21.
14. Zhang W, Mi J, Li N, et al. Identification and characterization of DPZF, a novel human BTB/POZ zinc finger protein sharing homology to BCL-6. Biochem Biophys Res Commun 2001;282:1067-73.
15. Mitchelmore C, Kjaerulff KM, Pedersen HC, et al. Characterization of two novel nuclear BTB/POZ domain zinc finger isoforms. Association with differentiation of hippocampal neurons, cerebellar granule cells, and macroglia. J Biol Chem 2002;277:7598-609.
16. Sutherland AP, Zhang H, Zhang Y, et al. Zinc finger protein Zbtb20 is essential for postnatal survival and glucose homeostasis. Mol Cell Biol 2009;29:2804-15.
17. Xie Z, Ma X, Ji W, et al. Zbtb20 is essential for the specification of CA1 field identity in the developing hippocampus. Proc Natl Acad Sci U S A 2010;107:6510-5.
18. Xie Z, Zhang H, Tsai W, et al. Zinc finger protein ZBTB20 is a key repressor of alpha-fetoprotein gene transcription in liver. Proc Natl Acad Sci U S A 2008;105:10859-64.
19. Herrera PL. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. Development 2000;127:2317-22.
20. Hashimoto N, Kido Y, Uchida T, et al. Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. Nat Genet 2006;38:589-93.
21. Carobbio S, Frigerio F, Rubi B, et al. Deletion of glutamate dehydrogenase in beta-cells abolishes part of the insulin secretory response not required for glucose homeostasis. J Biol Chem 2009;284:921-9.
22. Heo J, Factor VM, Uren T, et al. Hepatic precursors derived from murine embryonic stem cells contribute to regeneration of injured liver. Hepatology 2006;44:1478-86.
23. Zhou Y, Jetton TL, Goshorn S, et al. Transamination is required for {alpha}-ketoisocaproate but not leucine to stimulate insulin secretion. J Biol Chem 2010;285:33718-26.
24. Erion MD, van Poelje PD, Dang Q, et al. MB06322 (CS-917): A potent and selective inhibitor of fructose 1,6-bisphosphatase for controlling gluconeogenesis in type 2 diabetes. Proc Natl Acad Sci U S A 2005;102:7970-5.
25. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocr Rev 1998;19:491-503.

26. Butler AE, Janson J, Bonner-Weir S, et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102-10.
27. Deng S, Vatamaniuk M, Huang X, et al. Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. Diabetes 2004;53:624-32.
28. Del Guerra S, Lupi R, Marselli L, et al. Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 2005;54:727-35.
29. Gunton JE, Kulkarni RN, Yim S, et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 2005;122:337-49.
30. Cheng K, Ho K, Stokes R, et al. Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. J Clin Invest 2010;120:2171-83.
31. Doria A, Patti ME, Kahn CR. The emerging genetic architecture of type 2 diabetes. Cell Metab 2008;8:186-200.



基础医学开辟无限未来

杨书卷

（科技导报　北京　100081）

在现代医学中，洞悉生命和疾病现象本质及其规律的基础医学的重要性不言而喻，它的每一次突破，都会为直接医治病人的临床医学带来无限的治疗可能性。令人欣喜的是，在顶尖的基础医学领域，现在也时时闪动着中国科学家的身影。

6 月 29 日，英国 *Nature* 杂志以长文形式在线发表了一个让全世界顶尖生物学家都为之一震的惊人发现：中国科学家施一公领导的团队将阿尔茨海默病致病蛋白——人源 γ 分泌酶复合物进行了冷冻镜分析和数据收集，最终获得了分辨率高达 0.45 nm 的 γ 复合物三维结构，从其示意图中可以清晰地看到，“致病元凶”宛如一个静静蹲着的狮子。

2010 年的全球阿尔茨海默病患者人数约为 3080 万，我国目前的患者约占世界发病总数的 1/4，而随着老龄化进程的加速，到 2050 年，估计全球每 85 人就有 1 人罹患该病。据统计，阿尔茨海默病已是发达国家花费最高的疾病之一。

虽然在 10 多年前，科学家就知道了人源 γ 分泌酶复合物是阿尔茨海默病致病蛋白，但一直无法看清它到底“长成什么样”，这也是目前世界生命科学领域最热门的研究课题之一。在此之前，最好的“世界记录”是将其解析到 1.2 nm。“这好比是在 100 m 外看一个馒头。”施一公形象地对自己的研究成果做出了解释，“而我们现在做到的是在 5 m 外看一个馒头，未来的目标是做更精细解析，能在 10 cm 外看一个馒头。”

结构决定功能，要解释发病机理、进行药物研究，就必须先“看清”致病蛋白。早在 2004 年，施一公就锁定了这个结构生物学“制高点”，从细菌、酵母、昆虫细胞等多个表达系统中寻求突破，历经数年，最终利用瞬时转染技术在哺乳动物细胞中成功过量表达并纯化出致病蛋白，获得细微结构图，为世界科学界对阿尔茨海默病的研究填上了关键性的一环，被誉为在一场全球生命科学领域持续数十年最激烈赛跑中，中国科学家“完美撞线，领跑全球”。(7 月 10 日《科技日报》)

面对 21 世纪的超级癌症——艾滋病，近

期也出现了一种令人兴奋的新尝试：美国费城天普大学医学院的研究人员首次通过基因剪辑技术，从人体细胞中完全“删除”了艾滋病毒，这标志着人类朝永久治愈艾滋病迈出重要一步。

艾滋病毒最“恐怖”之处在于，一旦侵入人体细胞，就会在受害者 DNA 中永久植人致命基因，当今的艾滋病治疗只能达到“功能性”治愈，但不能彻底治愈，就是因为艾滋病病毒的基因组已经整合到病人细胞基因组中，永久侵害人体。研究负责人、神经科学系主任 Kamel Khalili 指出：“如需获得彻底根治，整合的潜伏病毒基因组就必须被完全根除”。

研究人员利用了近 1 年来极其热门的、被誉为“基因组编辑的魔术手术刀”的基因剪辑技术，就是通过 gRNA（核糖核酸）做向导，把一种叫做“Cas9”的剪切酶准确地带到相应的位置，然后用这种酶切割病毒 DNA，此后细胞可进行自我修复。在对艾滋病病毒潜伏感染的多种细胞模型进行实验中，包括巨噬细胞、小胶质细胞和 T 淋巴细胞等，均成功地根除了潜在的艾滋病病毒。

这项创新研究标志着首次成功从人体细胞中清除潜在的 HIV-1 病毒，同时，这种方法也有望应用于清除其他潜伏性感染病毒。不过，目前这一技术的临床应用还尚未完善，例如，如何把治疗物质输送到每一个感染细胞；此外艾滋病病毒易于变异，如何让治疗个性化，适应每个患者独特的病毒序列也是个问题。但正如 Khalili 所说：“它已经概念性地证明，我们正走在正确的方向上，未来根除每位患者体内的艾滋病毒的希望将逐步实现。”（7 月 14 日美国 *PNAS*)

在“闻之色变”的白血病治疗领域，美国贝勒医学院和得克萨斯儿童医院的科学家也找到了一种新的提高治疗存活率的有效方式。

骨髓移植是治疗白血病的有效方案，但患者在手术后一点小小的感染都有可能让一切前功尽弃。这是因为，在健康人的体内有一支 T 细胞军队在巡逻，随时预备着识别和对抗病毒，但白血病人在接受骨髓移植前为了防止排斥反应，却先要“抑制”自己的这一免疫系统，而移植的骨髓干细胞需要 4 个月到 1 年多的时间才能在受捐者体内扎根并促生新的免疫细胞，在此期间，病毒完全可以毫无屏障地“长驱直入”。虽然也可以从骨髓捐献者体内提取某些特定的抗病毒 T 细胞注人患者体内，但剂量必须进行定制，而且只能对抗一两种病毒，耗时则长达 3 个月。

而由 Ann Lien 带领的团队开发了一项制造 T 细胞的新技术，不仅速度快，对抗的目标病毒也增加到了最容易给患者惹麻烦的 5 种病毒，而且制造和冷冻经过设计的 T 细胞，前后耗时不过 10 天，相当于为患者撑起了一把方便、快捷，而且效果显著的巨大保护伞。研究团队下一步将尝试利用不同健康捐赠者的细胞创建一个“银行”，让所有病人都可以使用这种设计过的 T 细胞，免去定制剂量的繁琐，从而为众多患者带去福音(6 月 27 日中国科技网）。

7 月 24 日，一年一度的林道诺贝尔奖获得者大会聚焦医学和生理学，37 位在医学领域有突出贡献的诺贝尔奖获得者再一次分享了那些令人激动的伟大历史时刻：幽门螺杆菌才是胃溃疡的罪魁祸首；人乳头瘤病毒和宫颈癌之间密切相关；端粒酶的发现；“Toll 样受体”免疫系统……我们看到，医疗领域的绝大部分成果都来自这些基础研究。而在这个追求快速回报的时代，从事基础研究的科学家，尤其是年轻一代，虽然正受到各方面的压力，却不轻易放弃自己喜爱的课题，他们的坚守值得尊重和钦佩。

摘自《科技导报》第32卷第22期

解冻“渐冻人”

王丽娜
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近日，关于“冰桶挑战”的报道在媒体上铺天盖地。“冰桶挑战”源自美国，旨在让挑战者体会冰水浇头一刹那麻木无力的感觉，使更多人关注“渐冻人症”，并为他们筹得善款。“渐冻人症”又称肌萎缩侧索硬化症(ALS)，是一种运动神经元疾病，主要表现为肌肉逐渐无力、萎缩，进而说话、吞咽以及呼吸功能减退，直至因呼吸衰竭而死亡。“渐冻人症”是罕见疾病，患病率约十万分之五，目前对这种疾病还没有有效疗法，患者常在患病后 3~5 年内死亡，是世界五大绝症之一，杰出物理学家**霍金**因患这种病长期被束缚在轮椅上。近期，浙江大学求是高等研究院公布的脑机接口研究的成果为肢体行动非常不便的“渐冻人”带来了希望。

2 年前，浙江大学求是高等研究院的研究人员用猴子做脑机接口试验，将 2 个 4x4 mm 的芯片植人猴子大脑皮层中，使它们与 200 多个神经元相连接，进行神经信号采集，最终完成了“抓、勾、握、捍”这 4 个动作，实现了动物意念对机械手的控制。由于人脑受到外界或者思维的干扰较多，信号处理的复杂性也更大，直至 2014 年 8 月 25 日，研究人员宣布在动物实验的基础上，又实现了人脑对机械手的控制。

一位癫痫患者自愿接受这项实验， 她的大脑皮层下被埋人 32 个电极以便采集到更多、更准确的脑信号，然后再对记录的信号解码，最终将脑信号传递到机械手臂上。实验显示，患者可以顺利的完成“石头、剪刀、布”这 3 个较为复杂的手指动作，使得人脑控制机械手的能力达到了手指关节水平，控制动作的准确率约为 80% ；不仅如此，机械手还能判断患者的动作，与患者猜拳，并且每次都能赢（8 月 27 日《光明日报》）。

研究人员指出，脑机接口技术研究需要多学科交叉合作，研发出实时性强、准确性高、互适应功能好的多通道神经元放电采集、处理和信息解码技术是关键。目前，我国在脑机接口技术领域的研究处于世界前沿水平，如果这项研究成果得到广泛应用，无疑会给“渐冻人症”这种运动功能障碍的患者带来很大便利，使他们能够借助机械手臂自行完成正常人的肢体动作。

对于身体某些器官功能丧失的患者来说，如果也能够找到替代物来维持身体正常机能就再好不过了。目前，器官移植已经很普遍，但是，由于器官捐献者有限，需要器官移植的患者能够等到合适器官的几率仍然较小，很多患者不得不在等待中离世，于是科学家们尝试人 工培养活体器官，并在这方面取得很多可喜进展。近期发表在 *Nature Cell Biology* 上的研究成果显示，利用体外细胞成功在小鼠体内培育出完整的胸腺。

研究人员先将胚胎中提取出的纤维母细胞基因进行重组，转化成的细胞既有与胸腺细胞一样的外形，又能够支持 T 细胞发育。将重组后的细胞与其他起支撑作用的细胞一起放人小鼠体内，最后发育成功能齐全的胸腺（8 月 27 日《科技日报》）。

这是人类首次利用重组技术，将体外细胞培育成完整的活体器官。虽然在用于临床治疗之前仍然会面临种种不确定的因素或困难，但迈出的这一步却至关重要，它向我们描绘了一

幅愿景：仅将培养好的细胞注射进患者适当位置，便能长出适应人体的健全器官。

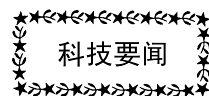
通常，疾病患者不仅要忍受肉体的痛苦，往往还忧心忡忡，担心是否能够痊愈、是否有其他并发症等等，这给患者带来严重的心理负担，在精神上也经受折磨。“渐冻人症”患者更是如此——他们的肢体不受控制，但具有清醒的意识，精神上的痛苦可想而知。如果痛苦的感觉能够淡化、清除或者能够被替代，或许会使他们生活得更轻松、愉快。27 日，*Nature* 上的一篇研究报告显示，情绪记忆是可塑的。

美国麻省理工学院的 Roger L. Redondo 等研究人员将小鼠分成 2 部分进行实验，一部分用电击使之形成恐惧记忆，另一部分通过与异性互动，使之得到正面记忆。研究人员分别记录了在这 2 种不同记忆形成过程中发挥作用的神经元。当小鼠再进人获得记忆的区域时，它们会出现条件反射：受电击的小鼠赶紧躲开，与异性互动的小鼠则会作停留。后来，研究人员采用光照来控制小鼠大脑的神经元活动，使它们的情绪记忆得以改写。结果表明，即使在与异性互动过的区域，当光照激活形成恐惧记忆的神经元时，小鼠会出现电击时的反应——赶紧躲开有关区域；相反，即使在电击时的区域，当激活形成美好记忆的神经元时，它们也会做出与异性互动时相似的反应。记忆是大脑系统思维活动的过程，当小鼠记忆被改写时，脑中负责编码情境信息的海马齿状回的回路被激活，从而改变了负责编码情绪信息的杏仁核区域与海马齿状回间的记忆连接（8 月 28 日新华网）。

此项研究表明，海马齿状回和杏仁核间的神经回路是改写情绪记忆的关键区域，也使我们相信人类情绪记忆的改写终有一天会成为现实。那时，不仅能减轻“渐冻人”的精神痛苦，也能为众多有心理障碍的人们扫去心中的阴霾。

科学的巨轮仍在滚滚前行，行过的地方泛起粼粼波光，那是“渐冻人”身体的“冰”融化的痕迹。期待不久的将来，“冰”会全部消融。

摘自《科技导报》第32卷第25期



对抗埃博拉 未来或迎持久战

2014 年 10 月 18 日消息，世界卫生组织（WHO）的最新数据显示，在受埃博拉疫情影响最严重的国家——利比里亚、几内亚和塞拉利昂，埃博拉疫情已导致 4546 人死亡，9191 人感染。

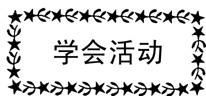
自 2014 年 3 月在西非暴发以来，埃博拉出血热这一致命性极高的流行病传播速度逐渐加快，造成的死亡人数不断升高。尼日利亚、塞内加尔和美国均出现感染者，有科学家甚至指出，埃博拉或于 10 月底传入英国和法国。此外还一度传出埃博拉病毒经变异后可通过空气传播的观点。埃博拉疫情形势严峻，正如美国疾控中心主任 Tom Friede 所说，对抗埃博拉病毒，“未来将会是一场持久战”。

摘自《科技导报》第 32 卷第 30 期

干细胞疗法，是契机也是考验

干细胞是一种尚未充分分化且不成熟的细胞，它具有再生成各种组织器官的潜在功能。目前很多疾病依靠传统医学手段难以攻克，干细胞疗法的产生和应用可以帮助病人缓解痛苦。许多科研人员认为，对于绝大多数疾病来说，包括神经性疾病、糖尿病、慢性心脏疾病、肾脏病、肝脏疾病、癌症和艾滋病等，都可望借助干细胞技术得到康复。尤其是获得 2012 年诺贝尔生理学或医学奖的日本科学家山中伸弥发现诱导多功能干细胞（iPS 细胞）之后，似乎让更多人看到了干细胞疗法的曙光。近期，又有不少关于干细胞的研究成果占据了新闻版面，但围绕着干细胞疗法的争议也同时成为新闻关注的焦点。干细胞治疗虽然可以被视为人类攻克疾病的契机，但也同时面临着考验——宗教因素与技术难题是干细胞治疗必须跨越的难关。

摘自《科技导报》第32卷第31期



四会齐开，百家争鸣，协同创新

——“缺氧”与“呼吸”的高峰论坛

冯丹丹

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2014 年 11 月 27-30 日，暑热还未消散的南方，一场关于“缺氧”和“呼吸”的盛大学术研讨会在广州医科大学热烈召开。本次会议包含了四个分会：“中华医学会第七次全国高原医学学术会议”、“呼吸重大疾病转化医学学术论坛”、“中国病理生理学会第九次全国缺氧和呼吸病理生理学术会议”以及“全国呼吸领域研究生论坛”。由中华医学会高原医学分会、中国生理学会呼吸专业委员会、中国病理生理学会缺氧和呼吸专业委员会和呼吸疾病国家重点实验室主办，第三军医大学高原军事医学系、呼吸疾病国家临床医学研究中心、广州呼吸疾病研究所及广州医科大学基础学院共同承办。据悉，紧凑的两天时间里，共有来自以上学术领域的专家或研究生分别在不同会场为 300 多名与会者奉上近 150 个专题的中文或英文学术报告。

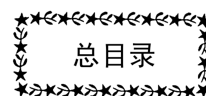
11 月 28 日上午，大会开幕式在广州医科大学东风校区礼堂举行。钟南山院士、王新华校长出席并致词。王新华校长代表广州医科大学欢迎来自全国各地高校的专家、学者，介绍了广医在教学、科研、医疗等各方面取得的成绩，希望通过本次会议推动高原医学的发展，促进各兄弟院校间的紧密合作，并祝愿会议取得圆满成功。钟南山院士致辞，指出本次会议的主题是“缺氧”和“呼吸”。多主题的分会可从不同角度来探索缺氧与呼吸生理，是一种值得提倡和推广的办会方式。此外，本次会议是一个基础和临床互相学习的平台，是来自全国各地的专家、学者、研究生学习、交流的机会。他希望大家充分交流，互相学习、协作。简短的开幕式后，立即进行大会报告。首先，全国高原

医学专家吴天一院士向大家介绍了各种高原工作环境下应对缺氧的不同习服方式及高原病的诊断和治疗。中国生理学会呼吸生理专业委员会主任委员罗自强教授、副主任委员胡清华教授分别在大会上作了“谷氨酸 NMDA 受体激活--一种新的肺损伤机制”和“线粒体异质性与肺动脉高压”的主题报告。此后各分会场的内容更是丰富多彩，涉及广泛，包括高原病、肺部炎症、肺纤维化、肺血管疾病，支气管哮喘等。

会议圆满闭幕，成功开创了多会齐办，学科交叉，百家争鸣的办会方式。会议精彩的报告，专家学者、研究生们热烈的讨论，密切的交流给大家留下了深刻的印象，也得到了所有与会者的高度评价，纷纷建议继续开展跨学会的专题学术交流，加强学科之间的交叉渗透，不断提升我国呼吸生理与病理生理学的研究水平。

呼吸会议通讯报道

2014 年 11 月 30 日



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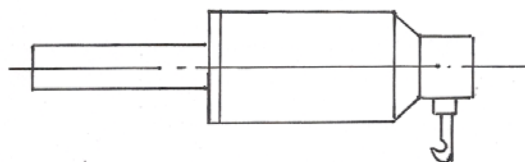

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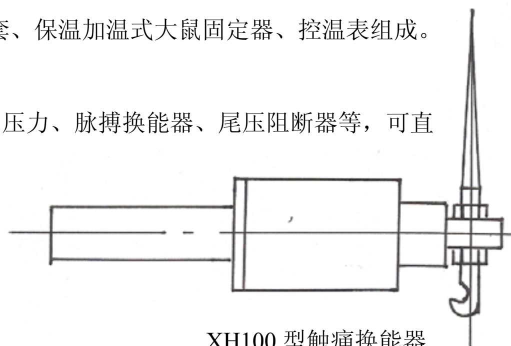
九、YP200 型压力换能器

主要是配国内外厂家生产的生物信号采集系统



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