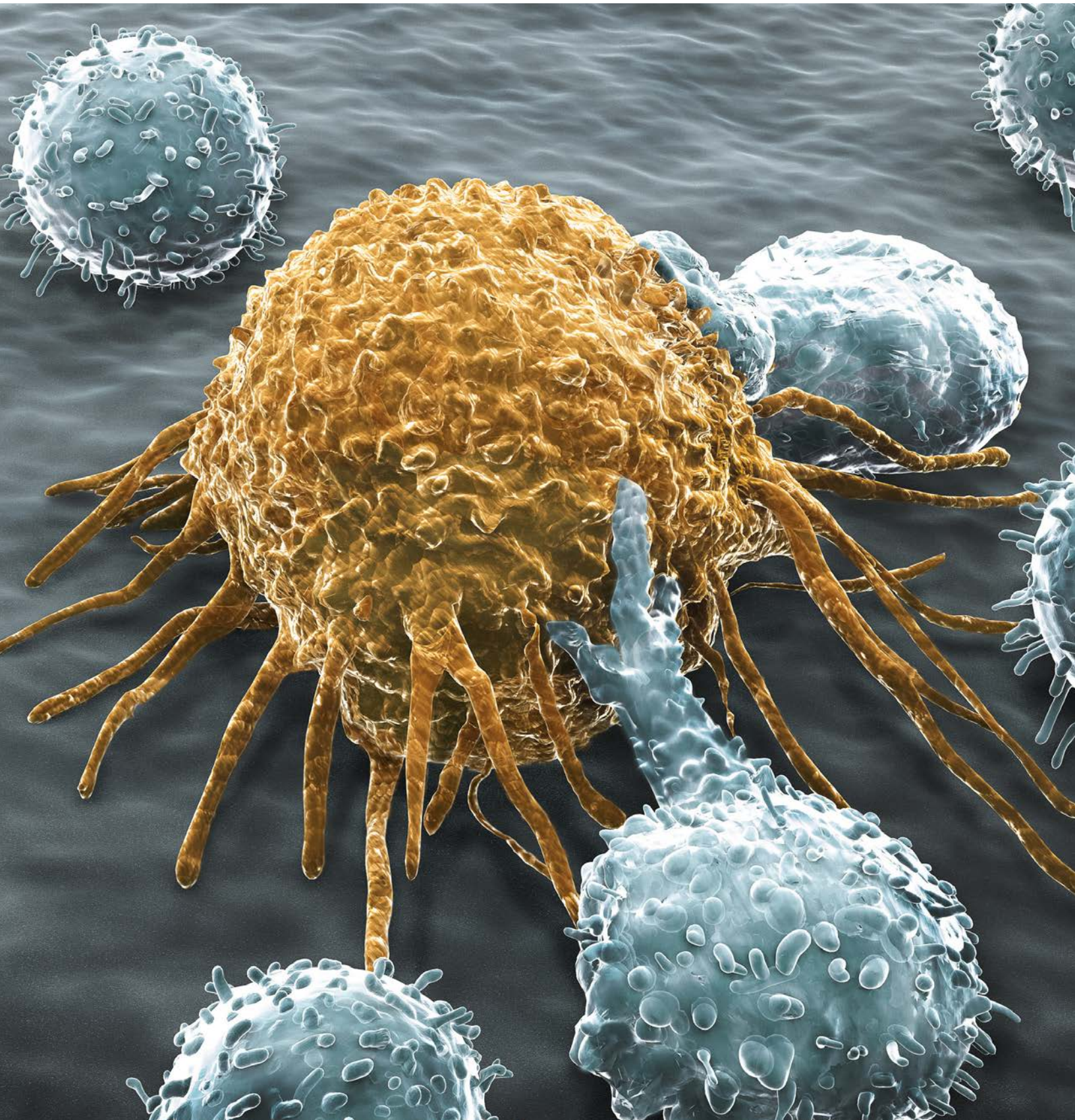
癌症和免疫系统

近期以Illumina®技术为特色的文献综述



目录

本文档着重突出了最近出版品中关于在免疫学研究中应用Illumina技术的部分。若有意深入了解文档中提到的平台和分析方法，请访问 www.illumina.com。

简介

免疫检查点抑制剂在对抗黑色素瘤中所获得的出色成果[1]，得益于几十年的免疫系统机制和控制的基础研究。这项研究建立了应对癌症治疗挑战的全套工具，包括能准确重复检查点抑制剂反应的小鼠模型，以应对癌症治疗的挑战。[2]具有讽刺意味的是，诸如ipilimumab[3]和nivolumab[4]的检查点抑制剂本身就是单克隆抗体。尽管目前的治疗主要集中在黑色素瘤，未必能让所有患者受益，但科学知识的坚实基础能让组合疗法和靶向疗法的使用有相对快速且合理的进展。[5]

高通量测序已经在癌症和免疫学研究以及个性化免疫疗法的开发上表现出显著的作用。例如，高通量测序已经大大改善了我们对癌症基因组以及肿瘤发展过程中细胞内机制的了解。此外，癌症基因组的精心分析也揭示了新的表位，它们有望成为免疫系统的目标。[6]测序还能确定免疫组库，从而高度灵敏地实时监控细胞群体在应对肿瘤生长或治疗时的克隆扩增和收缩。[7,8,9]

综述

Robinson W. H. (2015) Sequencing the functional antibody repertoire--diagnostic and therapeutic discovery. Nat Rev Rheumatol 11: 171-182

Chaudhary B., Abd Al Samid M., al-Ramadi B. K. and Elkord E. (2014) Phenotypic alterations, clinical impact and therapeutic potential of regulatory T cells in cancer.Expert Opin Biol Ther 14: 931-945

Giraldo N. A., Becht E., Remark R., Damotte D., Sautes-Fridman C., et al.(2014) The immune contexture of primary and metastatic human tumours.Curr Opin Immunol 27: 8-15

Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82

Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations.Curr Opin Immunol 27: 89-97

Rosenberg S. A. (2014) Finding suitable targets is the major obstacle to cancer gene therapy.Cancer Gene Ther 21: 45-47

1. Chapman P. B., D'Angelo S. P. and Wolchok J. D. (2015) Rapid eradication of a bulky melanoma mass with one dose of immunotherapy.N Engl J Med 372: 2073-2074
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12. Postow M. A., Chesney J., Pavlick A. C., Robert C., Grossmann K., et al.(2015) Nivolumab and ipilimumab versus ipilimumab in untreated melanoma.N Engl J Med 372: 2006-2017

免疫检查点抑制剂

使用有效策略阻断免疫抑制性受体正在彻底改变癌症治疗。[10]针对细胞毒性T淋巴细胞相关蛋白4（CTLA-4）、程序性细胞死亡受体1（PD-1）以及程序性细胞死亡1配体（PD-L1）的单克隆抗体可以抑制调节性免疫反应，产生明显且持久的反应。[11,12,13,14]然而，决定一位患者是否响应的因素还不清楚。[15,16]人们也研究了一些潜在的药物靶点，如磷脂酰肌醇3-激酶/蛋白激酶B（PI3K/AKT）通路[17,18]和溴结构域蛋白4（BRD4），[19]以改善响应（表1）。

> “讽刺的是，我们现在却担心可能产生过度强烈的抗黑色素瘤反应。” Chapman[20]

表1：免疫检查点抑制剂及其靶点。

名称	商品名称	目标	赞助商	参考文献
dabrafenib	Tafinlar	BRAF V600E 突变	GlaxoSmithKline (GSK)	21
vemurafenib	Zelboraf	BRAF V600E 突变	Daiichi-Sankyo	22
ipilimumab	Yervoy	CTLA-4	Bristol-Myers Squibb	23
tremelimumab	（未建立）	CTLA-4	Pfizer	24
pembrolizumab	Keytruda	PD-1	Merck & Co	25
nivolumab	Opdivo	PD-1	Bristol-Myers Squibb	26

13. Topalian S. L., Hodi F. S., Brahmer J. R., Gettinger S. N., Smith D. C., et al.(2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.N Engl J Med 366: 2443-2454
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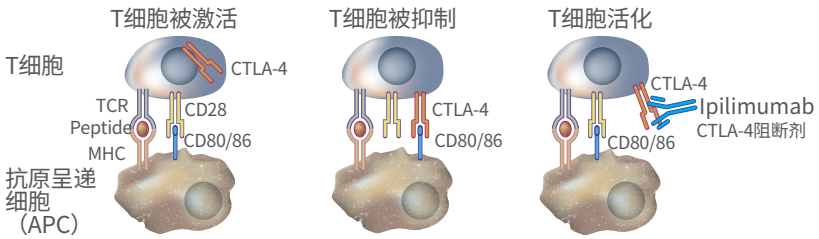
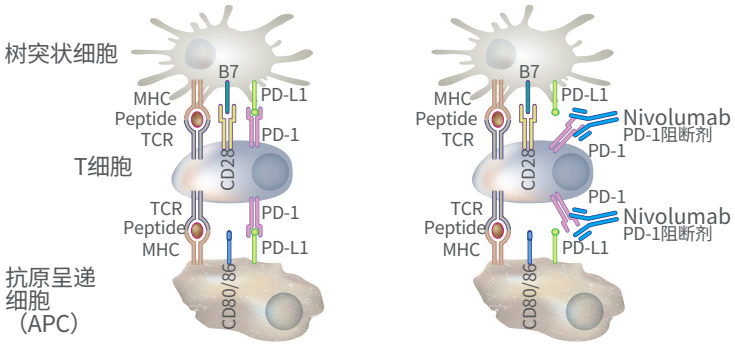


Ipilimumab的作用机制。Ipilimumab是一种单克隆抗体，它与CTLA-4结合，阻断CTLA-4与其配体CD80/CD86之间的相互作用。CTLA-4的阻断增加了T细胞的激活和增殖，这包括肿瘤浸润性T淋巴细胞的激活和增殖。(www.hcp.yervoy.com)



Nivolumab的作用机制。主要组织相容性复合物(MHC)/抗原的结合让T细胞识别肿瘤，使肿瘤细胞表面的PD-L1上调。PD-1/PD-L1的相互作用抑制了T细胞介导的肿瘤杀伤。PD-1/PD-L1相互作用的阻断再次激活T细胞介导的肿瘤杀伤。

综述

Leavy O. (2015) Tumour immunology: A triple blow for cancer.Nat Rev Immunol 15: 265

Chaudhary B., Abd Al Samid M., al-Ramadi B. K. and Elkord E. (2014) Phenotypic alterations, clinical impact and therapeutic potential of regulatory T cells in cancer.Expert Opin Biol Ther 14: 931-945

Gubin M. M., Zhang X., Schuster H., Caron E., Ward J. P., et al.(2014) Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens.Nature 515: 577-581

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Chen G., Chakravarti N., Aardalen K., Lazar A. J., Tetzlaff M. T., et al.(2014) Molecular profiling of patient-matched brain and extracranial melanoma metastases implicates the PI3K pathway as a therapeutic target.Clin Cancer Res 20: 5537-5546

ipilimumab和dabrafenib是FDA批准的治疗转移性黑色素瘤的两种新疗法，但它们在脑转移患者中的活性一般。作者比较了患者配对的大脑和颅外转移瘤，发现它们极其相似。然而，他们发现大脑转移瘤的可靶向通路有明显差异。特别是PI3K/AKT通路，它似乎在肿瘤激活中发挥关键作用。

Illumina的技术：HumanHT12 v4 BeadChip芯片

Kim K., Skora A. D., Li Z., Liu Q., Tam A. J., et al.(2014) Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells.Proc Natl Acad Sci U S A 111: 11774-11779

免疫原性差的癌症往往在免疫疗法上表现不佳。作者发现，对于耐受免疫检查点调节剂的小鼠，表观遗传调节药物和检查点抑制剂的共同治疗明显改善了治疗效果，治愈了80%以上含有肿瘤的小鼠。表观遗传调节剂的主要目标是髓源性抑制细胞（MDSC）。与免疫检查点抑制剂结合使用，降低循环MDSC的PI3K抑制剂也能清除80%小鼠中的4T1肿瘤。

Illumina的技术：Genome Analyzer$_{IIx}$和HiSeq

Robert L., Tsoi J., Wang X., Emerson R., Homet B., et al.(2014) CTLA4 blockade broadens the peripheral T-cell receptor repertoire.Clin Cancer Res 20: 2424-2432

作者分别在基线以及用tremelimumab阻断CTLA-4 30–60天后对21名患者的外周血单核细胞（PBMC）中的重排T细胞受体（TCR）可变β链的互补决定区3（CDR3）进行测序。他们发现CDR3序列的多样性增加，但这种多样性与临床响应者和非响应者并无关联。

Illumina的技术：Genome Analyzer

Snyder A., Makarov V., Merghoub T., Yuan J., Zaretsky J. M., et al.(2014) Genetic basis for clinical response to CTLA-4 blockade in melanoma.N Engl J Med 371: 2189-2199

作者对64名经过CTLA-4阻断治疗的恶性黑色素瘤患者的外显子组进行了测序，发现了强烈响应CTLA-4阻断的肿瘤特征。他们在第二组39名经过CTLA-4抗体治疗的黑色素瘤患者中验证了这种特征，发现预测的新抗原激活T细胞可以用ipilimumab治疗。

Illumina的技术：HiSeq 2000用于外显子组文库的测序

Tumeh P. C., Harview C. L., Yearley J. H., Shintaku I. P., Taylor E. J., et al.(2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance.Nature 515: 568-571

作者发现，位于侵袭性肿瘤边缘的预先存在的CD81 T细胞与PD-1/PD-L1免疫抑制性轴的表达相关联，有可能预测对治疗的反应。他们的结论是，在治疗性的PD-1阻断后，肿瘤消退需要预先存在的CD81 T细胞，它们受到PD-1/PD-L1介导的适应性免疫耐受的负向调节。

Illumina的技术：HiSeq

Rochman Y., Yukawa M., Kartashov A. V. and Barski A. (2015) Functional characterization of human T cell hyporesponsiveness induced by CTLA4-Ig.PLoS One 10: e0122198

Spranger S., Bao R. and Gajewski T. F. (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity.Nature 523: 231-235

Johnson D. B., Smalley K. S. and Sosman J. A. (2014) Molecular pathways: targeting NRAS in melanoma and acute myelogenous leukemia.Clin Cancer Res 20: 4186-4192

Sengsayadeth S., Wang T., Lee S. J., Haagenson M. D., Spellman S., et al.(2014) Cytotoxic T-lymphocyte antigen-4 single nucleotide polymorphisms are not associated with outcomes after unrelated donor transplantation: a center for international blood and marrow transplant research analysis.Biol Blood Marrow Transplant 20: 900-903

Westin J. R., Chu F., Zhang M., Fayad L. E., Kwak L. W., et al.(2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial.Lancet Oncol 15: 69-77

T细胞组库

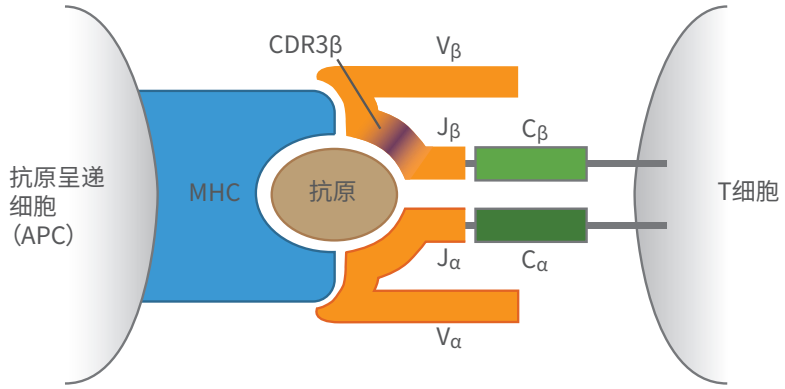
人体免疫系统为对抗各种各样的病原体以及肿瘤提供一种保护作用。这种保护是通过B细胞和T细胞表面与致病性或病原体来源的抗原结合的巨大的受体库来介导的。[27]T细胞通过表达异二聚体（αβ或γδ）细胞表面受体（T细胞受体，或TCR）来介导细胞免疫，这需要异源细胞呈递与MHC结合的肽段抗原。[28]

综述

Calis J. J. and Rosenberg B. R. (2014) Characterizing immune repertoires by high throughput sequencing: strategies and applications.Trends Immunol 35: 581-590

Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82

Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease.Genome Med 5: 98



T细胞受体-抗原-MHC相互作用以及T细胞受体（TCR）基因重排。a）抗原呈递细胞呈递与MHC结合的肽段抗原。TCR（橙色）与抗原和MHC结合。如果结合亲和力足够高，则T细胞被激活。互补决定区3（CDR3）结构域以紫色显示。

27. Robins H. (2013) Immunosequencing: applications of immune repertoire deep sequencing. Curr Opin Immunol 25: 646-652
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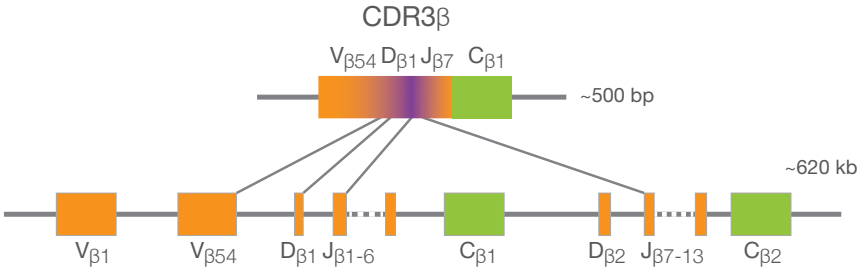
B细胞受体(BCR)和T细胞受体(TCR)中高度可变的CDR3区域很短,介于15–60个核苷酸之间,这使得它们特别适合新一代测序(NGS)。新一代测序已被广泛用于确定T群体。[29-32]在此过程中,TCR的β链通常作为一个标记。[33]



TCR-β VDJ基因重排导致TCR多样性的简化表示。TCR-β基因座位于7号染色体上,长度约为620 kb。最初两个D区域中的一个与13个J区域中的一个(两个都随机选择)连接,接着将DJ区域与50多个V区域中的一个(也是随机选择)连接,产生最终的VDJ区域,其长度约为500 bp。基因片段连接的机制也引入了碱基对的多样性,这与这些片段的组合选择一起带来TCR多样性。TCR-α链也经历了一个完全类似的过程,但不包括D基因片段。

传统技术(如流式细胞术[34]或谱型分析[35])的分辨率很低,不能分辨有着相同TCR-Vβ片段的TCR克隆型或相同长度的CDR3。[36,37]幸运的是,新一代测序技术能够确定特定T细胞群体中存在的所有TCRβ CDR3序列的核苷酸序列,即使它们以极低的频率存在。[38]由于TCRβ CDR3库的高度多样性,所获得的序列将在大多数情况下代表单个TCR克隆型。[39]新一代测序是一种客观的工具,能够准确测定T细胞群体,用于预后和监控治疗反应。[40]

功能性的TCR是由α和β链组成的异二聚体蛋白质。每个T细胞包含α和β链的独特组合,为了实现准确的功能分析,两个亚基都必须测序。为了避免细胞裂解对α和β链配对的破坏,[41]一些单细胞测序方法已经被开发出来。[42]关于更多信息,详见单细胞和TCR测序。

29. van Heijst J. W., Ceberio I., Lipuma L. B., Samilo D. W., Wasilewski G. D., et al.(2013) Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation.Nat Med 19: 372-377
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Qi Q., Liu Y., Cheng Y., Glanville J., Zhang D., et al.(2014) Diversity and clonal selection in the human T-cell repertoire.Proc Natl Acad Sci U S A 111: 13139-13144

作者利用新一代测序和非参数统计分析来估计人类组库中不同TCR-β序列总数的下限。他们估计，在年轻人的幼稚CD4和CD8 T细胞库中至少有一亿条独特的TCR-β序列。

Illumina的技术：MiSeq

Robert L., Tsoi J., Wang X., Emerson R., Homet B., et al.(2014) CTLA4 blockade broadens the peripheral T-cell receptor repertoire.Clin Cancer Res 20: 2424-2432

作者分别在基线以及用tremelimumab阻断CTLA-4 30–60天后对21名患者的外周血单核细胞（PBMC）中的重排TCR-Vβ链的CDR3区域进行测序。他们发现CDR3序列的多样性增加，但这种多样性与临床响应者和非响应者并无关联。

Illumina的技术：Genome Analyzer

Tumeh P. C., Harview C. L., Yearley J. H., Shintaku I. P., Taylor E. J., et al.(2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance.Nature 515: 568-571

作者发现，位于侵袭性肿瘤边缘的预先存在的CD81 T细胞与PD-1/PD-L1免疫抑制性轴的表达相关联，有可能预测对治疗的反应。他们的结论是，在治疗性的PD-1阻断后，肿瘤消退需要预先存在的CD81 T细胞，它们受到PD-1/PD-L1介导的适应性免疫耐受的负向调节。

Illumina的技术：HiSeq

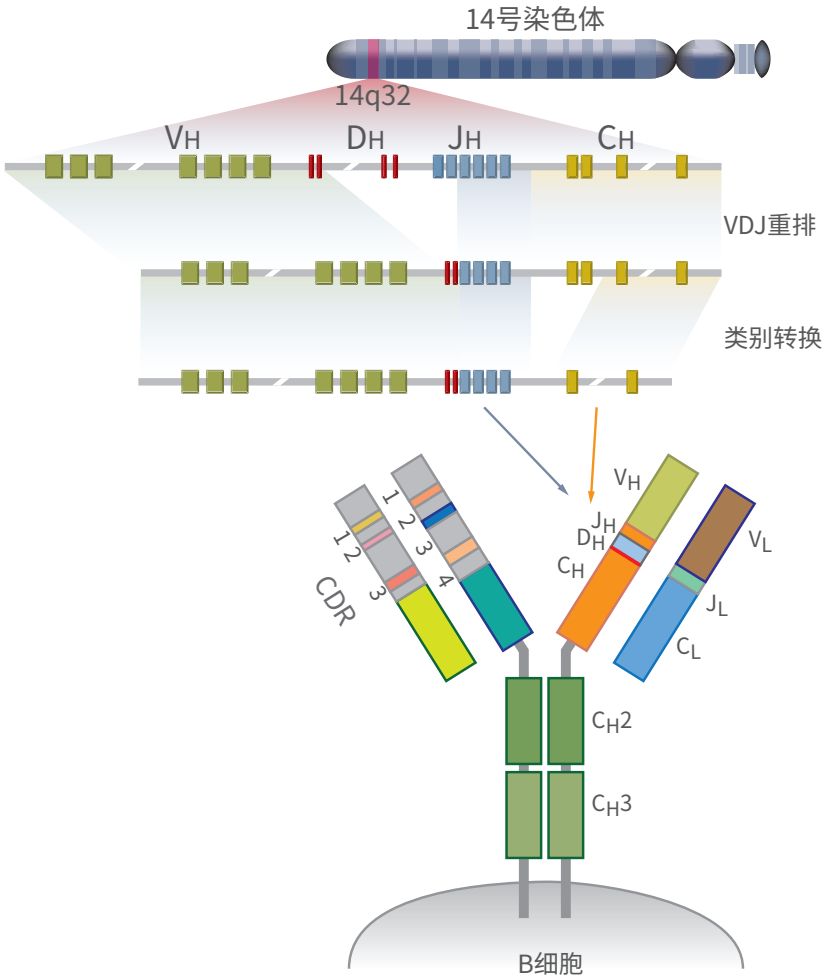
Bajor D. L., Xu X., Torigian D. A., Mick R., Garcia L. R., et al.(2014) Immune activation and a 9-year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma.Cancer Immunol Res 2: 1051-1058

Madi A., Shifrut E., Reich-Zeliger S., Gal H., Best K., et al.(2014) T-cell receptor repertoires share a restricted set of public and abundant CDR3 sequences that are associated with self-related immunity.Genome Res 24: 1603-1612

抗体库

抗体库测序正在改变我们对自身免疫、疫苗接种、感染和癌症的免疫反应的了解。这种技术有望为多种疾病（包括风湿病）带来新一代的生物标志物、诊断工具和治疗抗体。

43. Georgiou G., Ippolito G. C., Beausang J., Busse C. E., Wardemann H., et al.(2014) The promise and challenge of high-throughput sequencing of the antibody repertoire.Nat Biotechnol 32: 158-168



一抗的重链（IgH）库主要是通过VDJ基因片段的体细胞重组产生的。非模板的核苷酸（以红色表示）也被添加进去。重链的抗原结合位点是由高变的互补决定区（CDR-H1、H2和H3）和骨架区3（FR3）的并列形成的。在IgH重排之后，轻链（IgL）的重组跟着发生，而H和L链的异源二聚体配对形成了完整的IgM同型抗体，它们在新形成的不成熟B细胞表面表达。[43]

综述

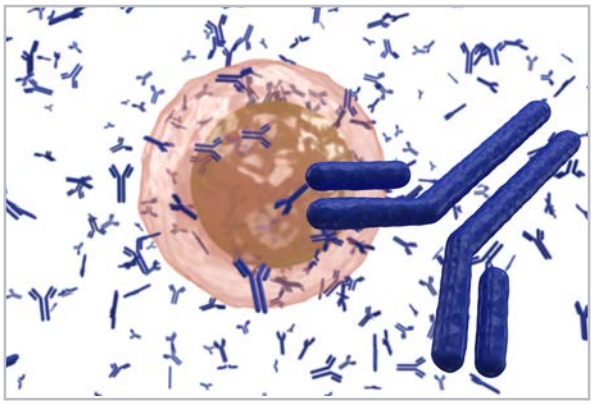
Robinson W. H. (2015) Sequencing the functional antibody repertoire--diagnostic and therapeutic discovery. Nat Rev Rheumatol 11: 171-182

Calis J. J. and Rosenberg B. R. (2014) Characterizing immune repertoires by high throughput sequencing: strategies and applications.Trends Immunol 35: 581-590

Dheilly N. M., Adema C., Raftos D. A., Gourbal B., Grunau C., et al.(2014) No more non-model species: the promise of next generation sequencing for comparative immunology.Dev Comp Immunol 45: 56-66

Georgiou G., Ippolito G. C., Beausang J., Busse C. E., Wardemann H., et al.(2014) The promise and challenge of high-throughput sequencing of the antibody repertoire.Nat Biotechnol 32: 158-168

Shugay M., Britanova O. V., Merzlyak E. M., Turchaninova M. A., Mamedov I. Z., et al.(2014) Towards error-free profiling of immune repertoires.Nat Methods 11: 653-655



B细胞产生的抗体。

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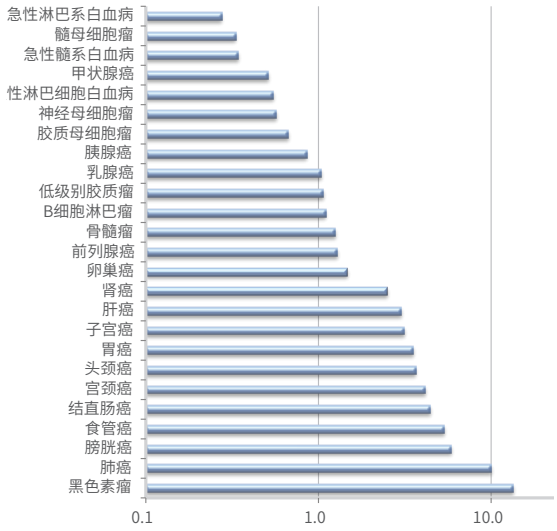
Birnbaum M. E., Mendoza J. L., Sethi D. K., Dong S., Glanville J., et al.(2014) Deconstructing the peptide-MHC specificity of T cell recognition.Cell 157: 1073-1087

Menzel U., Greiff V., Khan T. A., Haessler U., Hellmann I., et al.(2014) Comprehensive evaluation and optimization of amplicon library preparation methods for high-throughput antibody sequencing.PLoS One 9: e96727

癌症表位

由于广泛的遗传和表观遗传改变，肿瘤细胞产生了大量正常细胞中不存在的蛋白质。这些蛋白质导致MHC I类相关肽段库的改变。表位的范围不仅包括肿瘤细胞中异常表达的基因所产生的肽段，还包括因肿瘤细胞中的体细胞突变而产生的“新抗原”。因此，这些新抗原是肿瘤特异的，也是患者独有的。T细胞可识别人类肿瘤细胞表面的这些抗原，从而介导癌症消退。[44,45]

最近大量使用新一代测序来鉴定癌症基因组，为鉴定潜在的肿瘤特异抗原谱提供了一个独特的机会。[46,47,48]动物模型[49,50]以及人类癌症[51,52,53]的外显子组测序数据，可以预测T细胞对肿瘤特异突变所形成的新抗原的反应。人们逐步了解哪些抗原在有效免疫疗法中形成了主要目标，最终有望带来更准确的预后和治疗。[54,55]



各种人类癌症中体细胞突变的发生率。[56]

44. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens.Curr Opin Immunol 25: 284-290
45. Walter S., Weinschenk T., Stenzl A., Zdrojowy R., Pluzanska A., et al.(2012) Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival.Nat Med 18: 1254-1261
46. Garraway L. A. and Lander E. S. (2013) Lessons from the cancer genome.Cell 153: 17-37
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50. Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al.(2012) Exploiting the mutanome for tumor vaccination.Cancer Res 72: 1081-1091
51. Robbins P. F., Lu Y. C., El-Gamil M., Li Y. F., Gross C., et al.(2013) Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 19: 747-752
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53. van Rooij N., van Buuren M. M., Philips D., Velds A., Toebes M., et al.(2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma.J Clin Oncol 31: e439-442
54. Heemskerk B., Kvistborg P. and Schumacher T. N. (2013) The cancer antigenome.EMBO J 32: 194-203
55. Lu Y. C., Yao X., Li Y. F., El-Gamil M., Dudley M. E., et al.(2013) Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression.J Immunol 190: 6034-6042
56. Alexandrov L. B., Nik-Zainal S., Wedge D. C., Aparicio S. A., Behjati S., et al.(2013) Signatures of mutational processes in human cancer.Nature 500: 415-421

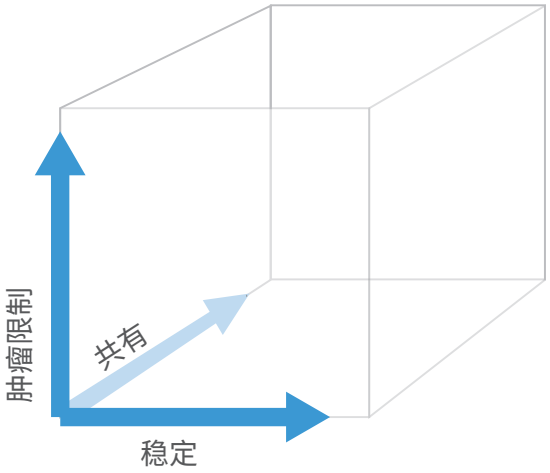
综述

Rosenberg S. A. (2014) Finding suitable targets is the major obstacle to cancer gene therapy.Cancer Gene Ther 21: 45-47

开发具有抗肿瘤活性的淋巴细胞已经成为目前癌症免疫疗法研究中的一项主要工作。然而，作为靶点的癌细胞表面蛋白在健康组织上仍有表达，尽管水平很低，却会导致治疗存在严重毒性作用的风险。主要的障碍是如何确定癌细胞上适当的免疫目标。对于遗传改造的淋巴细胞，理想的抗原是共有的突变，它们是每种癌症类型特有的，且不出现在正常组织上。例如，假设适当的抗原受体能被鉴定出的话，黑色素瘤中的B-RAF或胰腺癌及其他癌症中的K-RAS等常见突变就可能代表了细胞转移免疫疗法的理想靶点。淋巴细胞的基因编辑为这个基因治疗领域开辟了新的潜能。

Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens.Curr Opin Immunol 25: 284-290

细胞毒性T细胞可以识别人类肿瘤细胞表面的抗原，从而介导癌症消退。为了利用癌症治疗的潜能，挑战仍在于鉴定肿瘤抗原，它们需要满足：i)为患者组所共有；ii)只在肿瘤中表达；iii)在选择性压力下抗原丢失的可能性低。随着新一代测序的发展，人们能够相对轻松地描述个体肿瘤中肿瘤特异的突变库，就有可能预测满足这些标准的患者突变抗原。



人类的肿瘤相关抗原特征的3D表示。稳定：在T细胞压力下抗原保留的可能性；肿瘤限制：与正常相比，肿瘤独特性的程度；共有：患者之间共有的程度。[57]

Heemskerk B., Kvistborg P. and Schumacher T. N. (2013) The cancer antigenome.EMBO J 32: 194-203

Haen S. P. and Rammensee H. G. (2013) The repertoire of human tumor-associated epitopes--identification and selection of antigens and their application in clinical trials.Curr Opin Immunol 25: 277-283

van Rooij N., van Buuren M. M., Philips D., Velds A., Toebes M., et al.(2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma.J Clin Oncol 31: e439-442

57. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens.Curr Opin Immunol 25: 284-290

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Green M. R., Kihira S., Liu C. L., Nair R. V., Salari R., et al.(2015) Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation.Proc Natl Acad Sci U S A 112: E1116-1125

作者利用新一代测序来重建22名患者的滤泡性淋巴瘤活检中的突变层次。他们发现，与同一肿瘤的野生型B细胞相比，CREBBP突变的B细胞较少刺激体外T细胞增殖。转录特征显示肿瘤浸润性CD4辅助性T细胞和CD8记忆细胞毒性T细胞的数量减少。

Illumina的技术：HiSeq 2000

Kreiter S., Vormehr M., van de Roemer N., Diken M., Lower M., et al.(2015) Mutant MHC class II epitopes drive therapeutic immune responses to cancer.Nature 520: 692-696

这项研究描述了通过外显子组测序选择哪些突变作为疫苗靶点的过程，这只是根据它们的表达水平以及MHC II类分子的结合能力进行的生物信息学优先排序。这一信息可用于快速生产多个新表位的信使RNA（mRNA）合成疫苗。作者表明，这种“多表位”mRNA疫苗的接种在小鼠中诱导了有效的肿瘤控制以及不断增长肿瘤的完全抑制。

Illumina的技术：HiSeq 2000用于外显子组和mRNA测序

Yadav M., Jhunjhunwala S., Phung Q. T., Lupardus P., Tanguay J., et al.(2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing.Nature 515: 572-576

作者开发了一种鉴定新表位的方法，它综合了使用质谱法进行的全外显子组和转录组测序分析。小鼠的疫苗接种确认了这种方法，其中每条预测的免疫原性肽段都产生了治疗上有效的T细胞反应。这种方法可用于T细胞反应的药效监控，以及癌症患者的个性化疫苗开发。

Illumina的技术：HiSeq 2000

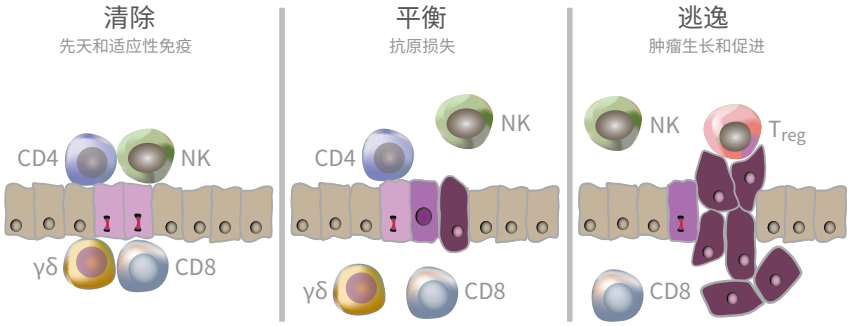
van Buuren M. M., Dijkgraaf F. E., Linnemann C., Toebes M., Chang C. X., et al.(2014) HLA micropolymorphisms strongly affect peptide-MHC multimer-based monitoring of antigen-specific CD8+ T cell responses.J Immunol 192: 641-648

Robbins P. F., Lu Y. C., El-Gamil M., Li Y. F., Gross C., et al.(2013) Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells.Nat Med 19: 747-752

van Rooij N., van Buuren M. M., Philips D., Velds A., Toebes M., et al.(2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma.J Clin Oncol 31: e439-442

肿瘤免疫编辑

肿瘤免疫编辑是适应性和先天免疫系统控制肿瘤生长和塑造肿瘤免疫原性的过程。[58,59,60,61,62]此过程包括三个阶段：清除（elimination）、平衡（equilibrium）和逃逸（escape）。[63]清除，或肿瘤免疫监控，是指适应性和先天免疫分支鉴定和破坏新形成的癌细胞的过程。平衡是最长的阶段，包括防止肿瘤生长和塑造少量瘤细胞的免疫原性之间的平衡状态。在逃逸阶段，最少免疫原性的肿瘤细胞逐渐生长并扩散为可见的肿瘤。



免疫编辑既防止又促进肿瘤生长。清除阶段描述了当组织中出现肿瘤时，适应性和先天免疫反应识别和清除肿瘤的过程。平衡阶段包括了防止肿瘤生长和选择不被杀死的癌细胞之间的平衡状态。这个阶段的结果是肿瘤细胞的定向选择，它们不再表达外来抗原，也不再表达MHC。逃逸阶段指的是变异的肿瘤细胞逃避免疫系统的清除机制和/或招募调节细胞来保护它们的过程。

外显子组测序让研究人员能够从实验上鉴定肿瘤表位，并具体鉴定未编辑肿瘤的表位。最近一项研究已经证明了该过程，该研究将大规模并行测序与cDNA捕获测序（cDNA CapSeq）技术相结合。结果表明，T细胞依赖的免疫编辑是缺乏强排斥抗原的肿瘤细胞增殖的潜在机制。[64]

58. Schreiber R. D., Old L. J. and Smyth M. J. (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion.Science 331: 1565-1570
59. Dunn G. P., Koebel C. M. and Schreiber R. D. (2006) Interferons, immunity and cancer immunoediting.Nat Rev Immunol 6: 836-848
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61. Dunn G. P., Old L. J. and Schreiber R. D. (2004) The three Es of cancer immunoediting.Annu Rev Immunol 22: 329-360
62. Shankaran V., Ikeda H., Bruce A. T., White J. M., Swanson P. E., et al.(2001) IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity.Nature 410: 1107-1111
63. Mittal D., Gubin M. M., Schreiber R. D. and Smyth M. J. (2014) New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape.Curr Opin Immunol 27: 16-25
64. Matsushita H., Vesely M. D., Koboldt D. C., Rickert C. G., Uppaluri R., et al.(2012) Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting.Nature 482: 400-404

综述

Mittal D., Gubin M. M., Schreiber R. D. and Smyth M. J. (2014) New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape.Curr Opin Immunol 27: 16-25

Vogelstein B., Papadopoulos N., Velculescu V. E., Zhou S., Diaz L. A., Jr., et al.(2013) Cancer genome landscapes.Science 339: 1546-1558

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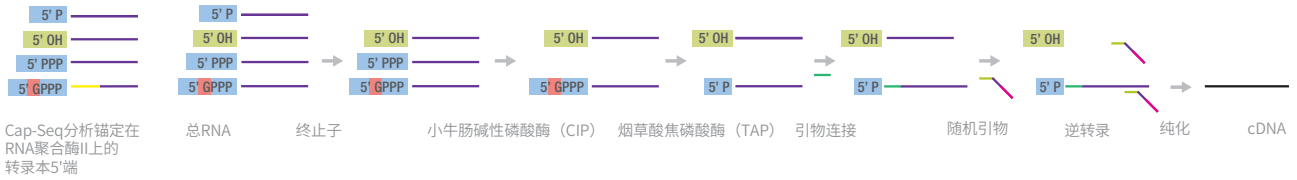
65. Illingworth R. S., Gruenewald-Schneider U., Webb S., Kerr A. R., James K. D., et al.(2010) Orphan CpG islands identify numerous conserved promoters in the mammalian genome. PLoS Genet 6: e1001134

Rooney M. S., Shukla S. A., Wu C. J., Getz G. and Hacohen N. (2015) Molecular and genetic properties of tumors associated with local immune cytolytic activity.Cell 160: 48-61
作者开发出一种基于RNA的免疫细胞杀伤活性指标，并计算出数千个癌症基因组图谱（TCGA）实体瘤样本的数值。他们发现，DNA扩增与高的细胞杀伤活性相关联，包括免疫抑制因子PDL1/2和ALOX12B/15B。他们也发现了免疫编辑的证据。

Illumina的技术：Genome Analyzer和HiSeq的数据

Cap-Seq



CXXC亲和纯化测序（CAP-Seq）[65]定位了锚定在RNA聚合酶II上的RNA的5'端。在这种方法中，RNA转录本先经过终止子、小牛肠碱性磷酸酶（CIP）处理，再经过烟草酸焦磷酸酶（TAP）处理，与接头连接，并逆转录为cDNA。cDNA的深度测序带来了高分辨率的RNA聚合酶II转录本序列。

肿瘤微环境

肿瘤微环境的定义是肿瘤存在的细胞环境。这包括周围的血管、免疫细胞、成纤维细胞、其他细胞、信号分子以及细胞外基质（ECM）。研究也报道了肿瘤与微环境之间的动态关系。这包括肿瘤通过释放细胞外信号（即肿瘤血管生成）而控制微环境，以及微环境发挥促进癌细胞生长的作用，如免疫编辑（更多详情请看《肿瘤免疫编辑》）。即使在转移病灶中，肿瘤微环境也还是一个主要的预后因素，同时在原发性和转移性肿瘤之间可以再生。尽管如此，根据原发性肿瘤的来源，Th1/细胞毒性T细胞浸润的预后影响是不同的。[66]

新的证据表明，可能存在肿瘤的不同亚群，反映出来便是不同类别的免疫逃逸。例如，缺乏趋化因子介导的运输，先天免疫细胞的激活不佳，以及存在特定的免疫抑制机制，可以用来鉴定肿瘤的亚群。

目前也有一些综合的发现，展示了慢性炎症建立促肿瘤微环境的不同机制。炎性反应可增加细胞应答信号，加速细胞周期，这反过来增加突变率，并最终促进肿瘤生长。[67]

综述

Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations.Curr Opin Immunol 27: 89-97

免疫治疗剂为开发癌症疗法带来了新的机会。免疫系统细胞上的抑制性受体（检查点）可被抑制剂靶向检测，以加强对癌细胞的免疫应答。单克隆抗体（mAb）就属于这一类的检查点抑制剂。一些单克隆抗体已在临床试验中得到应用，而一些研究也表明了单克隆抗体与化疗和放疗的组合策略的潜力。

Giraldo N. A., Becht E., Remark R., Damotte D., Sautes-Fridman C., et al.(2014) The immune contexture of primary and metastatic human tumours.Curr Opin Immunol 27: 8-15

Gajewski T. F., Woo S. R., Zha Y., Spaapen R., Zheng Y., et al.(2013) Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment.Curr Opin Immunol 25: 268-276

Galon J., Angell H. K., Bedognetti D. and Marincola F. M. (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures.Immunity 39: 11-26

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Zhou P., Shaffer D. R., Alvarez Arias D. A., Nakazaki Y., Pos W., et al.(2014) In vivo discovery of immunotherapy targets in the tumour microenvironment.Nature 506: 52-57

为了发现组织微环境中免疫功能的调节剂，作者开发了一种体内短发夹RNA（shRNA）筛选。靶向检测阴性调节剂的shRNA在识别肿瘤抗原时会在鼠类肿瘤中大量富集。结果表明，肿瘤中Ppp2r2d的敲落抑制了T细胞凋亡，增进了T细胞增殖和细胞因子的产生。

Illumina的技术：Genome Analyzer

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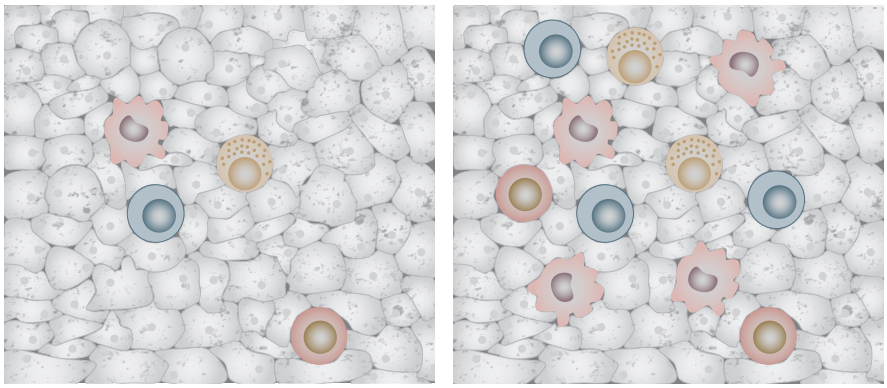
瘤内T细胞

T细胞对人体肿瘤的浸润是一种常见现象。人们已经观察到，浸润的程度和瘤内T细胞群体的反应性可以预测疾病的进程和结果。[68]为了利用这一发现，自体肿瘤浸润性淋巴细胞（TIL）以及淋巴细胞删除性预处理方案后的IL-2已用于治疗转移性黑色素瘤患者。[69]这种方法可使得20–40%的转移性黑色素瘤患者的癌症消退，其中大多数是现有方案难以治愈的。[70]为了扩展这一方法，人们需要鉴定甚至修饰TIL以及它们的靶点。[71]

初步实验表明，新一代测序可用来确定肿瘤内的免疫细胞群体的数量。[72]



T细胞对人体肿瘤的浸润是一种常见现象。而瘤内T细胞群体的特征可预测疾病的进程和结果。

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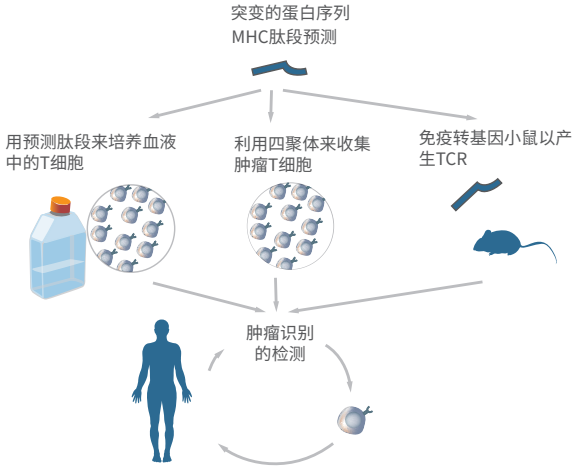
肿瘤免疫疗法

瘤内T细胞已被用于过继T细胞治疗的临床研究。[73]对于转移性黑色素瘤患者，基于肿瘤浸润淋巴细胞（TIL）的过继转移可导致肿瘤缩小50%。[74]相反，缺乏TIL被认为是免疫疗法效果不佳的一个标志。[75]两种机制可能在治疗耐受上起作用：因炎症水平低而缺乏T细胞迁移以及明显的免疫抑制。利用细胞因子白介素2（IL-2）来治疗，刺激T细胞的生长和增殖，已经在黑色素瘤和肾癌患者中产生了可持续较长时间的疗效，但不幸的是，这只在部分患者上有效。[76]



高度个性化的医疗。对患者肿瘤所表达的基因进行测序，以鉴定候选的突变T细胞表位。可通过至少三种方式中的一种来使用来自突变蛋白的肽段。首先，表达相关抗原的细胞可利用四聚体之类的试剂进行分选。第二，候选肽段可用来刺激已经存在于患者肿瘤或外周血中的T细胞。第三，抗原可用于引发人源化小鼠中肿瘤特异的T细胞，如果它们是人源的，还可过继转移。[77]

肿瘤的免疫原性来自产生肿瘤特异性抗原（TSA）的突变。这是大部分癌症的共同特点，但并非全部。[78]然而，靶向检测TSA带来了肿瘤特异性这个好处，降低了诱导自身免疫反应的风险。它还能靶定驱动突变，通过抗原损失来阻止肿瘤逃逸。高通量测序可以快速鉴定单个肿瘤内的突变，通过计算预测最能刺激T细胞反应的肽段，并让患者接种针对他们肿瘤内独特TSA的疫苗。[79,80,81]

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74. Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer.Curr Opin Immunol 27: 46-52
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基于树突状细胞（DC）的肿瘤疫苗耐受性良好，副作用少。可产生抗肿瘤的免疫应答，但从总体而言，它们的好处仍然有限。最近的研究表明，CD141+ DC在抗肿瘤反应中发挥重要作用。直接针对体内DC的疫苗目前正在开发中。[82]

82. Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy.Curr Opin Immunol 27: 26-32

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Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations.Curr Opin Immunol 27: 89-97

免疫治疗剂为开发癌症疗法带来了新的机会。免疫系统细胞上的抑制性受体（检查点）可被抑制剂靶向检测，以加强对癌细胞的免疫应答。单克隆抗体（mAb）就属于这一类的检查点抑制剂。一些单克隆抗体已在临床试验中得到应用，而一些研究也表明了单克隆抗体与化疗和放疗的组合策略的潜力。

Choi W., Porten S., Kim S., Willis D., Plimack E. R., et al.(2014) Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy.Cancer Cell 25: 152-165

Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer.Curr Opin Immunol 27: 46-52

Hinrichs C. S. and Rosenberg S. A. (2014) Exploiting the curative potential of adoptive T-cell therapy for cancer.Immunol Rev 257: 56-71

Nishikawa H. and Sakaguchi S. (2014) Regulatory T cells in cancer immunotherapy.Curr Opin Immunol 27: 1-7

Couzin-Frankel J. (2013) Breakthrough of the year 2013.Cancer immunotherapy.Science 342: 1432-1433

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Galon J., Angell H. K., Bedognetti D. and Marincola F. M. (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures.Immunity 39: 11-26

Hutchinson E. (2014) Towards individualized cancer therapy: Challenges and prospects.Molecular Oncology 8: 1-8

Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens.Curr Opin Immunol 25: 284-290

Phan G. Q. and Rosenberg S. A. (2013) Adoptive cell transfer for patients with metastatic melanoma: the potential and promise of cancer immunotherapy.Cancer Control 20: 289-297

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为了评估瘤内CD4+ T细胞对肿瘤中的非同义体细胞突变的反应，作者利用全外显子组测序和RNA测序数据来鉴定所表达基因中的一整套肿瘤特异的非同义突变。这种癌症中的CD4+新抗原反应主要针对个体突变，这让它们成为个性化免疫疗法的有力候选。

Illumina的技术：HiSeq 2000

Garralda E., Paz K., Lopez-Casas P. P., Jones S., Katz A., et al.(2014) Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment.Clin Cancer Res 20: 2476-2484

为了鉴定可行的肿瘤特异性基因改变，作者对25名晚期实体瘤患者开展了全外显子组测序分析。在14名患者中成功建立了10个小鼠异体移植（Avatar）模型。这些Avatar模型中候选治疗的检测与临床反应相关，可以帮助一些没有可行突变的患者选择经验疗法。

Illumina的技术：HiSeq 2000

Yadav M., Jhunjhunwala S., Phung Q. T., Lupardus P., Tanguay J., et al.(2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing.Nature 515: 572-576

作者开发了一种鉴定新表位的方法，它综合了使用质谱法进行的全外显子组和转录组测序分析。小鼠的疫苗接种确认了这种方法，其中每条预测的免疫原性肽段都产生了治疗上有效的T细胞反应。这种方法可用于T细胞反应的药效监控，以及癌症患者的个性化疫苗开发。

Illumina的技术：HiSeq 2000

Zhou P., Shaffer D. R., Alvarez Arias D. A., Nakazaki Y., Pos W., et al.(2014) In vivo discovery of immunotherapy targets in the tumour microenvironment.Nature 506: 52-57

作者表明，利用短发夹RNA（shRNA）筛选可以发现体内的治疗靶点。通过这种方法，他们鉴定出能改变荷瘤小鼠中肿瘤浸润性CD8 T细胞行动的基因。

Illumina的技术：Genome Analyzer

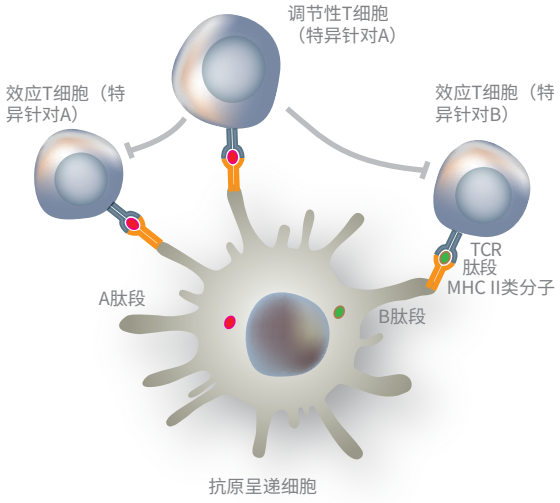
Bajor D. L., Xu X., Torigian D. A., Mick R., Garcia L. R., et al.(2014) Immune activation and a 9-year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma.Cancer Immunol Res 2: 1051-1058

Robbins P. F., Lu Y. C., El-Gamil M., Li Y. F., Gross C., et al.(2013) Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells.Nat Med 19: 747-752

树突状细胞

树突状细胞（DC）调控免疫反应，在一些癌症的清除中发挥作用。它们已作为疫苗开发的靶点，但鲜有成功。如今人们知道，不成熟的DC通常诱导耐受，而不是刺激免疫，因此大多数试验如今融入了Toll样受体（TLR）的配体和/或细胞因子来特异地激活DC。DC亚群在位置、表型和功能上也有差异。对这些复杂性的深入了解也许最终能带来更高效的基于DC的癌症疫苗。[83]

树突状细胞和淋巴细胞，着色的扫描电镜照片。



树突状细胞是重要的抗原呈递细胞，能够呈递多种抗原。它们是特别高效的辅助性T细胞活化剂，但连锁抑制也代表了调节性T细胞（TREG）支持自身耐受性的一种方式。TREG细胞抑制抗原呈递细胞（APC）呈递它们的同源抗原。它们也通过可溶性的抑制因子来抑制相同和不同抗原特异性的旁观T细胞。

83. Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy.Curr Opin Immunol 27: 26-32

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Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer.Curr Opin Immunol 27: 46-52

Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy.Curr Opin Immunol 27: 26-32

Kawakami Y., Yaguchi T., Sumimoto H., Kudo-Saito C., Iwata-Kajihara T., et al.(2013) Improvement of cancer immunotherapy by combining molecular targeted therapy.Front Oncol 3: 136

Tesone A. J., Svoronos N., Allegrezza M. J. and Conejo-Garcia J. R. (2013) Pathological mobilization and activities of dendritic cells in tumor-bearing hosts: challenges and opportunities for immunotherapy of cancer. Front Immunol 4: 435

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单核细胞来源的传统树突状细胞（ConvDC）因ConvDC难以生产和效力低而受到阻碍。在这项研究中，作者证明了慢病毒载体（LV）编程的DC的高效力。Illumina的MiSeq被用于质量检查和整合位点验证。

Illumina的技术：MiSeq

Ma Y., Mattarollo S. R., Adjemian S., Yang H., Aymeric L., et al.(2014) CCL2/CCR2-dependent recruitment of functional antigen-presenting cells into tumors upon chemotherapy.Cancer Res 74: 436-445
蒽环类药物用于癌症化疗的疗效依赖于树突状细胞的诱导以及T淋巴细胞依赖的抗癌免疫反应。这项研究探讨了小鼠癌症模型中以蒽环类药物为基础的化疗对趋化因子CCL2及其受体CCR2的影响。作者利用Illumina的Mouse BeadArray来鉴定基因表达差异。他们发现，基于蒽环类药物的化疗促进了肿瘤内骨髓细胞的累积，包括介导抗原呈递的细胞。这些发现加深了我们对免疫原性细胞死亡所诱发的抗癌免疫反应的了解。

Illumina的技术：Mouse WG-6 V.2 Expression BeadChips

Shalek A. K., Satija R., Adiconis X., Gertner R. S., Gaublomme J. T., et al.(2013) Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells.Nature 498: 236-240

Su X., Qian C., Zhang Q., Hou J., Gu Y., et al.(2013) miRNomes of haematopoietic stem cells and dendritic cells identify miR-30b as a regulator of Notch1.Nat Commun 4: 2903

血液肿瘤

从正常造血细胞向癌细胞的发展涉及到由一系列体细胞突变所驱动的克隆演化多步骤过程。这些突变逐渐将细胞从正常生长转化成癌前状态以及最终的癌状态，而意在调控细胞生长的所有检查点都被越过。

恶性转化的诱导似乎至少有两个不同的阶段：启动和促进。启动涉及到基因组的改变，但它本身不导致恶性转化。恶性转化需要第二个步骤，称为促进。促进发生在启动阶段后的侵袭性细胞分裂过程中。由新的DNA改变累积而引起，通常影响原癌基因、肿瘤抑制基因或凋亡基因，导致细胞生长不受调控。

新一代测序通过深度测序检测稀有克隆类型或细胞中的突变的能力，让它能够研究免疫效应功能在血液肿瘤发病中的作用。有一个已经大量报道的明显例子，认为克隆性干细胞疾病的发病与自身反应性T细胞克隆有关，如骨髓增生异常综合征（MDS）和再生障碍性贫血（AA）。[84]人们认识到由T细胞介导的抗肿瘤免疫力的破坏，容易发展成血液肿瘤，这也是对以上研究的一种佐证。总的来说，这些T细胞库研究以及涉及到急性淋巴性白血病的克隆演化中免疫球蛋白重链重排的新报道已迅速成为血液学中最令人兴奋的研究领域之一。[85,86,87]

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86. Gawad, C., Pepin, F., Carlton, V. E. H., Klinger, M., Logan, A. C., et al.(2012) Massive evolution of the immunoglobulin heavy chain locus in children with B precursor acute lymphoblastic leukemia.Blood 120: 4407-4417
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人们可采用高通量测序，以前所未有的灵敏度和特异性检测恶性B和T细胞中携带的CDR3序列重排。例如，在诊断时可通过IGH [88-94]和TCRβ/γ [95]基因的测序来鉴定B-和T-淋巴瘤患者中的恶性克隆。在后续的治疗中及治疗后，这一信息可用来追踪恶性克隆。对儿科急性B淋巴细胞白血病（B-ALL）中IGH位点的连续测序发现了某些患者中位点的惊人动态演化，[92]证明这种技术也能为了解B-和T-细胞癌症的生物学提供宝贵线索。

抗原-受体位点的深度测序为监控淋巴瘤带来了不少好处。与其他方法相比，深度测序耗时耗力更少，有着出色的灵敏度，[92,93,95]可同时追踪恶性群体中包含的所有克隆。这种测序在监控慢性淋巴细胞白血病、[89,90,91]儿科B谱系ALL[92,93]和T谱系ALL[95]患者的疾病负荷方面的作用已经得到证明，无疑在监控其他恶性淋巴瘤上也有作用。

综述

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Dose M., Emmanuel A. O., Chaumeil J., Zhang J., Sun T., et al.(2014) beta-Catenin induces T-cell transformation by promoting genomic instability.Proc Natl Acad Sci U S A 111: 391-396

癌变细胞的特点是细胞调控机制的功能异常，导致生长不受控制。在某些癌症中，这种调控异常导致基因组的不稳定性，例如会发生恶性重组事件。这项研究探索了β-连环蛋白的失调和基因组不稳定性之间的关联。作者研究了一个β-连环蛋白靶向激活的小鼠模型，并利用染色质免疫沉淀测序（ChIP-Seq）来确定转录因子结合位点和易位位点之间的关联。作者总结道，β-连环蛋白促进了基因组不稳定性，从而导致T细胞淋巴瘤。

Illumina的技术：Genome Analyzer$_{II}$

Joseph C. G., Darrah E., Shah A. A., Skora A. D., Casciola-Rosen L. A., et al.(2014) Association of the autoimmune disease scleroderma with an immunologic response to cancer.Science 343: 152-157

硬皮病是一种结缔组织的自身免疫性疾病，其中患者对有限的一组自身抗原产生抗体。硬皮病以及带有RPC1抗体的患者的癌症风险增加。作者对16名患者的POLR3A、TOP1和CENPB基因的肿瘤和正常编码序列进行测序。结果表明，POLR3A突变引发了细胞免疫和交叉反应性的体液免疫应答。

Illumina的技术：Genome Analyzer$_{IIx}$

Palomero T., Couronne L., Khiabanian H., Kim M. Y., Ambesi-Impiombato A., et al.(2014) Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas.Nat Genet 46: 166-170

外周T细胞淋巴瘤（PTCL）是异质性的，属于我们了解不多的非霍奇金淋巴瘤。在这项研究中，作者利用全外显子组测序来分析12对肿瘤–正常的DNA样本组，接着利用Illumina的HiSeq 2000进行RNA测序，并利用Illumina的MiSeq进行深度靶向重测序来验证已鉴定出的遗传变异。他们鉴定出新的和复发的遗传缺陷，包括FYN、ATM、B2M和CD58中的突变，SRC信号受损，DNA损伤反应受损以及PTCL从免疫监视机制中逃逸。

Illumina的技术：HiSeq 2000、MiSeq

Papaemmanuil E., Rapado I., Li Y., Potter N. E., Wedge D. C., et al.(2014) RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia.Nat Genet 46: 116-125

至少有四分之一的急性淋巴性白血病（ALL）病例含有ETV6-RUNX1融合基因。尽管这个基因融合是疾病的特征，但还需要其他突变，才能发展成症状明显的白血病。这项研究利用外显子组以及低覆盖度的全基因组测序来鉴定与白血病转化相关的次级事件。作者发现ATF7IP和MGA是ALL中两个新的肿瘤抑制基因，并简单描述了将ETV6-RUNX1阳性的淋巴母细胞转化成白血病的突变过程。

Illumina的技术：Genome Analyzer

Sakata-Yanagimoto M., Enami T., Yoshida K., Shiraishi Y., Ishii R., et al.(2014) Somatic RHOA mutation in angioimmunoblastic T cell lymphoma.Nat Genet 46: 171-175

血管免疫母细胞性T细胞淋巴瘤（AITL）是外周T细胞淋巴瘤（PTCL）的一种独特亚型。这项研究探索了这个淋巴瘤亚型特有的分子特征。利用Illumina HiSeq和MiSeq开展全外显子组、靶向测序和RNA测序，作者鉴定出肿瘤细胞中存在的RHOA体细胞突变。他们认为，RHOA功能受损与之前TET2功能丧失结合，形成了AITL的发病机理。

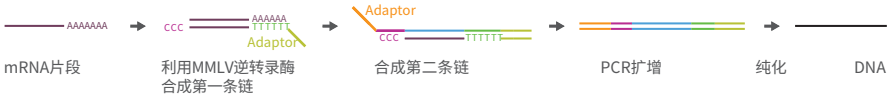
Illumina的技术：MiSeq和HiSeq 2000，100 bp read

Sherwood A. M., Emerson R. O., Scherer D., Habermann N., Buck K., et al.(2013) Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue.Cancer Immunol Immunother 62: 1453-1461

单细胞和TCR测序

功能性的TCR是异源二聚体蛋白，包含α和ß链。每个T细胞包含α和β链的独特组合，为了实现准确的功能分析，两个亚基都必须测序。为了避免细胞裂解对α和β链配对的破坏，[96]一些单细胞测序方法已经被开发出来。[97]

Smart-Seq



Smart-Seq作为一种单细胞测序方法，可改善转录本的序列覆盖。[98]在这个操作中，细胞被裂解，而RNA与含有oligo(dT)的引物杂交。之后产生第一条链，并添加几个无模板的C核苷酸。接着，寡核苷酸引物与poly(C)垂悬杂交，合成第二条链。全长cDNA经过PCR扩增，获得纳克水平的DNA。PCR产物经过纯化，用于测序。[99]

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TCR链配对

基于细胞的油包水RT-PCR技术可鉴定TCR α-β链配对。释放的TCRα和β mRNA经过逆转录，扩增，并在每个液滴中重叠延伸。从油包水乳液中提取产物，对感兴趣的融合分子进行选择性扩增。未融合的分子被阻断性引物抑制。[100]

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Ma Y., Mattarollo S. R., Adjemian S., Yang H., Aymeric L., et al.(2014) CCL2/CCR2-dependent recruitment of functional antigen-presenting cells into tumors upon chemotherapy.Cancer Res 74: 436-445

蒽环类药物用于癌症化疗的疗效依赖于树突状细胞的诱导以及T淋巴细胞依赖的抗癌免疫反应。这项研究探讨了小鼠癌症模型中以蒽环类药物为基础的化疗对趋化因子CCL2及其受体CCR2的影响。作者利用Illumina的Mouse BeadArray来鉴定基因表达差异。他们发现，基于蒽环类药物的化疗促进了肿瘤内骨髓细胞的累积，包括介导抗原呈递的细胞。这些发现加深了我们对免疫原性细胞死亡所诱发的抗癌免疫反应的了解。

Illumina的技术：小鼠（基因表达—BeadArray）

Papaemmanuil E., Rapado I., Li Y., Potter N. E., Wedge D. C., et al.(2014) RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia.Nat Genet 46: 116-125

至少有四分之一的急性淋巴性白血病（ALL）病例含有ETV6-RUNX1融合基因。尽管这个基因融合是疾病的特征，但还需要其他突变，才能发展成症状明显的白血病。这项研究利用外显子组以及低覆盖度的全基因组测序来鉴定与白血病转化相关的次级事件。作者发现ATF7IP和MGA是ALL中两个新的肿瘤抑制基因，并简单描述了将ETV6-RUNX1阳性的淋巴母细胞转化成白血病的突变过程。

Illumina的技术：Genome Analyzer

Bolotin D. A., Shugay M., Mamedov I. Z., Putintseva E. V., Turchaninova M. A., et al.(2013) MiTCR: software for T-cell receptor sequencing data analysis.Nat Methods 10: 813-814

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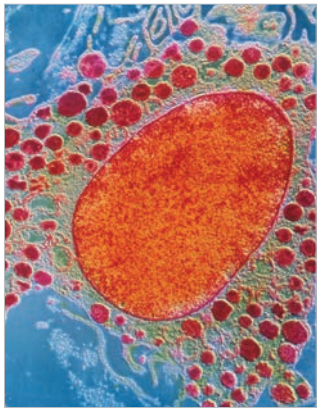

免疫学研究综述

免疫组库测序让研究人员能够从易患恶性血液病、自身免疫疾病和过敏原反应的个体中鉴定出独特的受体变异。

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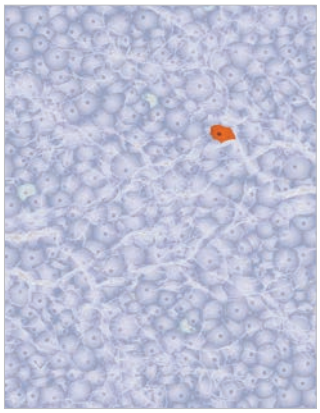

单细胞研究综述

单细胞组织测序的主要动力来自癌症研究，其中细胞谱系和残留疾病的检测是我们最关心的问题。我们正利用相同的方法，来改善我们对高度复杂的生物学系统的了解，比如神经发育和免疫学。

这篇文档着重突出了最近发表的一些文献，它们证明了Illumina技术在单细胞测序以及极低起始量的应用和技术中的使用。

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