CURRICULUM VITAE

Full name:	Qianqian Gao
Mobile:	(+86) 15201158281
Email:	gaoqianqian@genomics.cn; gauk_g@163.com
Mailing address:	Jinsha Road, Dapeng New District, Shenzhen, 518083, China

QUALIFICATIONS

A molecular immunologist with a strong knowledge in molecular cloning, epigenetics of rDNA transcription, CAR- and TCR-T cell engineering, CRISPR/Cas9-based next generation cell immunotherapy and single-cell sequencing.

- Focused on epigenetic regulations including histone methylation and acetylation of rDNA transcription during Ph.D. training.
- Three years' experience in cell immunotherapy to establish platform for CAR-T cell immunotherapy, develop and explore next generation CAR-T and TCR-T technology using CRISPR/Cas9 gene editing tool, and investigate the role of PD-1 and its inhibition in fighting against cancer.

TECHNICAL SKILLS

- **Molecular Biology:** Molecule cloning, ChIP (Chromatin immunoprecipitation), Real-time PCR, DNA/RNA/protein extraction, Western Blot, siRNA transfection, DNA electrophoresis, TEM (Transmission Electron Microscope), LSCM (Laser scanning Confocal Microscopy).
- **Cell Biology and Immunology:** CRISPR/Cas9 gene editing, Lentivirus packing and infection, Flow cytometry and cell sorting (FACS), PBMC or T cell preparation from human blood, Cell lines and primary T cells culturing, Cell transfection, DNA/RNA/RNP electroporation, Cell immunofluorescence staining, CCK-8 assay, Colony formation assay, Cell proliferation tests (cell counting, BrdU incorporation, CFSE incorporation), Apoptosis assay, CAR-T/TCR-T cell manufacturing, Cell cytotoxicity test, Cell cytokine tests (ELISA, ELISPOT) and single-cell RNA sequencing.
- **Animal experiments:** Drosophila melanogaster rearing and crossing, brain isolation, heat-shock assay; C.elegans rearing and crossing, lifespan test.

EDUCATION

2016-2020	Research scientist /Master supervisor /Postdoctoral fellow	Immunotherapy/ Anti-tumor immunology	Institute of Precision Oncology, BGI, Shenzhen, China
2010-2016	Ph.D.	Cell biology	School of Life Sciences, Peking University, Beijing, China

2006-2010	Bachelor	Biotechnology	Marine College, Shandong University, Weihai, China

AWARDS and HONORS

2019	Lecturer of the tenth free micro-course of CNGBdb
2019	BGI Women Speaker Award of ICG-14 conference, Shenzhen, China
2019	Excellent Poster Award, BGI
2019	Master Instructor of BGI College
2018	Major Project Contribution Award, BGI
2017	Category D "Phoenix Tree" Talent of Yantian, Shenzhen
2016	Outstanding graduates in Beijing, Outstanding graduates of Peking University
2015	Excellent Student Awards of Peking University, Founder Scholarship for Excellent Student of Life Science, Peking University
2013	Social Work Award of Peking University
2006-2010	5.4 Youth Science Award of Shandong University, First Prize of "Challenge Cup" of Shandong Province (first author), National Scholarship, three consecutive years, Provincial Top Students Award of Shandong Province, Top Ten Students Award in Shandong University, First Class Scholarship and Excellent Student Awards of Shandong University, four consecutive years

RESEARCH EXPERIENCE

- **Ongoing projects 1: The role of PD-1/PD-L1 signaling and its disruption in immunotherapy** (Leader)

The role of PD-1/PD-L1 signaling in TCR-T immunotherapy is now explored through high-throughput single-cell 3’ mRNA transcriptome sequencing (scRNA-seq), multiplex cytokine secretion assay, together with cell cytotoxicity assay. Our results will reveal the single-cell level transcriptional features, as well as cytokine and cytotoxic signatures of TCR-T cells upon antigen-specific stimulation under different expression ratio of PD-L1 molecules on target cells.

At the same time, PD-1 is eliminated by CRISPR/Cas9 and its function will be further studied by single-cell sequencing. In addition, PD-1 disruption by gene editing will be compared with PD-1 antibody blockade in TCR-T cell function and differentiation, which will provide reference for clinical therapy.

- **Ongoing projects 2: TIL therapy for hepatocellular carcinoma (HCC)** (Leader)

A new opened project. HCC samples will be provided by the cooperated hospital and TIL and tumor cells will be isolated from the tumor samples. TIL features will be characterized by different biomarkers and single-cell sequencing. On another hand, antigen/neoantigen presentation will be

detected by MS and the whole-exome of tumor cells will be sequenced to find the candidate neoantigens. TIL will be stimulated by the potential neoantigens to discover the TCR sequences and their uniqueness will be characterized.

- **Research experience 1: Distinct immune response landscapes of COVID-19 and influenza patients revealed by single-cell sequencing** (Main participant)

PBMCs were collected in both COVID-19 patients and influenza A virus-infected patients and the transcriptome landscape of them was investigated by single-cell RNA sequencing. We find dramatic changes in specific immune cells during infection and identified the factors/events associated with cell composition and activation status, featured with a leaping of plasma cells and *XAF1*- and *TNF*-induced apoptosis in the role of T lymphocyte reduction. By comparative analysis between COVID-19 and IAV patients, we found distinct signaling pathways that are activated in COVID-19 (STAT1 and ISG 15) and IAV (STAT3 and NFκB) patients. we further identified a substantial difference in the expression of key factors, including elevated expression of IL-6R and IL-6ST in COVID-19 patients, which supports the clinical observations on cytokine storm.

- **Research experience 2: Targeting oncogenic *KRAS* G12S mutant allele by CRISPR/Cas9 induces efficient tumor regression** (Leader)

KRAS is one of the most frequently mutated oncogenes in cancers. The protein's picomolar affinity for GTP/GDP and smooth protein structure resulting in the absence of known allosteric regulatory sites makes its genomic-level activating mutations a difficult but attractive target. Two CRISPR systems, genome-editing CRISPR/SpCas9 and transcription-regulating dCas9-KRAB, were developed to deplete the *KRAS* G12S mutant allele or repress its transcription, respectively, with the goal of treating *KRAS*-driven cancers. SpCas9 and dCas9-KRAB systems with a sgRNA targeting the mutant allele blocked the expression of the mutant *KRAS* gene, leading to an inhibition of cancer cell proliferation. Local adenoviral injections using SpCas9 and dCas9-KRAB systems suppressed tumor growth in vivo. The gene-depletion system (SpCas9) performed more effectively than the transcription-suppressing system (dCas9-KRAB) on tumor inhibition. Application of both Cas9 systems to wild-type *KRAS* tumors did not affect cell proliferation. Furthermore, through bioinformatic analysis of 31555 SNP mutations of the top 20 cancer driver genes, the data showed that our mutant-specific editing strategy could be extended to a reference list of oncogenic mutations with high editing potentials. This pipeline could be applied to analyze the distribution of PAM sequences and survey the best alternative targets for gene editing.

- **Research experience 3: Developing next-generation CAR-T and TCR-T cell immunotherapy using CRISPR/Cas9 system** (Leader)

CAR-T cell therapy is a promising approach by engineering patients' own lymphocytes to treat advanced cancers. In order to reduce susceptibility of CAR-T cells to immunosuppressive tumor microenvironments and improve persistence and anti-tumor activity of CAR-T cells against tumors, I have knocked out inhibitory signaling receptors such as PD-1, CTLA-4 and VISTA separately or in combination by CRISPR/Cas9 gene editing in human primary T cells. Gene-edited T cells secreted more IFNγ and their anti-tumor activity remains to be further explored next. On the other hand, in order to improve the ratio of both CAR-positive and checkpoint gene-knockout T cells, I constructed a system by incorporating multiple gRNAs driven by different promoters in a CAR lentiviral vector. The gene editing efficiency was confirmed and the cloning method was applied for a patent [2]. Currently, we are utilizing these platforms to edit TCR-T cells to improve the anti-tumor efficacy.

- **Research experience 4: Establishing anti-CD19 CAR-T platform and preclinical study of anti-BCMA CAR-T** (Leader)

As CAR-T cell immunotherapy is shown efficient against hematologic malignancies in clinical trials, I applied anti-CD19 CAR-T as a PoC sduty to build our own CAR-T platform. Different anti-CD19 CAR lentiviral constructs were cloned with different regulatory elements or gene fragments. The results showed different CAR constructs led to various expression level of CAR, so as to the level of IFNγ secretion. After establishment of anti-CD19 CAR-T platform, anti-BCMA CAR-T was also investigated and its preclinical study was finished.

- **Research experience 5: Building antigen-processing tool cell line without endogenous HLA gene backgrounds for mass spectrometry (MS) detection** (Leader)

To establish a cell line without endogenous HLA gene backgrounds for neoepitope prediction of neoantigen-based immunotherapy, we knocked out HLA-C gene in C1R B lymphoma cell line using CRISPR/Cas9 genome editing system. A master HLA class I-null cell line has been generated. Besides, other HLA-type C1R could be established by overexpressing a given HLA gene using this master HLA-null cell line. One patent [3] was applied based on this work. Another T2 tool cell line was also established in the same way for peptide binding.

PATENTS and SOFTWARE COPYRIGHT

[1]. Chao C#, **Gao Q**, Han X, Hou Y, Wang F, Li B, Li Y, Ze B. One therapy for KRAS mutation-driven tumor based on CRISPR/Cas9. 2017-11-13, P2017-1-0169.CN.

[2]. **Gao Q**#, Chao C, Li Y, Ding R, Li B, Hou Y, Wang F. Establishing methods of different promoters-driven sgRNAs expression plasmids. 2018-08-16, P2018-1-0121.CN.

[3]. Chao C#, **Gao Q**, Duan K, Wang F, Ding R, Xu Q, Dong X, Zhu L, Ge Y, Li Bo, Hou Y. Establishing methods of antigen-processing cell line without endogenous HLA gene backgrounds. 2018-08-09, P2018-1-0161.WO.

[4]. Huang L, Wang D, **Gao Q**, Jiang Y, Shen Y. Off-target rate calculation pipeline (OTRCP). 2019-11

PUBLICATIONS

= co-first authors; * = corresponding authors

Zhu L#, Yang P#, Zhao Y#, Zhuang Z#, Wang Z#, Song R#, Zhang J#, Liu C, **Gao Q**, Xu Q, Wei X, Sun H, Ye B, Wu Y, Zhang N, Gong Y, Lei G, Yu L, Yan J, Diao G, Yu Y, Wang M, Yuan Y, Deng Q, Li Z, Huang Y, Hu G, Xu Z, Liu P, Bi Y, Shi Y, Chen Z, Wang J, Xu X, Wu G, Zhang S, Gao G F, Liu L*, Liu W J*. Distinct immune response landscapes of COVID-19 and influenza patients revealed by single-cell sequencing. **New England Journal of Medicine** (**IF=70.67**, under review)

Gao Q#*, Ouyang W#, Kang B#, Han X#, Ding R, Li Y, Wang Fei, Huang L, Chen L, Wang D, Dong X, Zhang Z, Li Y, Ze B, Hou Y, Yang H, Ma Y*, Gu Y*, Chao C*. Selective targeting of the oncogenic KRAS G12S mutant allele by CRISPR/Cas9 induces efficient tumor regression. **Theranostics**, 2020; 10(11):5137-5153. doi:10.7150/thno.42325. (**IF=8.063**)

Gao Q#, Dong X, Xu Q, Zhu L, Wang F, Hou Y, Chao C*. Therapeutic Applications of CRISPR/Cas9 Gene Editing in Engineered T Cell-based Cancer Immunotherapy. **Cancer Medicine**,

2019, 8(9): 4254-4264. DOI: 10.1002/cam4.2257 (**IF=3.357**)

Wang F#, Xu Q#, Zhuang Z#, Liu S, Li Z, **Gao Q**, Huang Y, Chen C, Li Y, Zhang W, Liu L, Hou Y, Gu Y*, Zhang X*, Zhu L*, Cheng C*. Rapid generation of TCR engineered T lymphocytes by linking the single-cell transcriptome to its corresponding T-cell receptor in antigen-specific T cells. **Science Translational Medicine** (**IF=17.161**, submitted)

Huang X#, Zong L, **Gao Q**, Zhang C, Zhang L, Lyu G*, Tao W*. Gene body methylation safeguards ribosomal DNA transcription via preventing PHF6-mediated H4K20me3 enrichment. **PLoS Genetics** (**IF=5.224**, under review)

Ding R#, Liu S#, Wang S#, Chen H, Xu Q, Dong X, Gu Y, Zhu L*, Chao C C*, **Gao Q***. Single-cell transcriptome reveals a landscape of PD-L1 inhibition on anti-tumor efficacy of MART-1 TCR-T cells. (Manuscript in preparation)

Kang B#, **Gao Q#**. Multiplex editing of KRAS and PI3K pathways effectively blocks the progression of KRAS mutated colorectal cancer. (Manuscript in preparation)

Shen M#, Zhou T, Xie W, Ling T, Zhu Q, Zong L, Lyu G, **Gao Q**, Zhang F, Tao W*. The Chromatin Remodeling Factor CSB Recruits Histone Acetyltransferase PCAF to rRNA Gene Promoters in Active State for Transcription Initiation, **PLOS One**, 2013. (**IF=3.337**)

Gao Q#, Zhu Q*. LMS and its application, **Environmental Engineering**, 2009. (in Chinese)

Gao Q#, Zhu Q, Yang L, Yang Z. Decolorization and Degradation of Dispersed Blue-2BLN by Immobilized Fungal Laccase, **Sichuan Environment**, 2009. (in Chinese)

MEETING ABSTRACT

1. Ding R., Chen H, Xu Q, Zhu L, Dong X, Gu Y, Chao C-C* **Gao Q***. Enhancing anti-tumor efficacy of MART-1-specific TCR-T cells by CRISPR-Cas9-based PD-1 disruption. Permanent Abstract Number: 2204. AACR Annual Meeting 2020 in San Diego, California, America.

PARTICIPATED GRANTS

Dong X#, **Gao Q**, Chao C, Ye L, Wang S. Plug-in and Run Antibody-coupled CAR-T Platform of Emerging Cancer Immunotherapy for the treatment of colorectal cancer. 2018.1.1-2019.12.31. Free exploring basic research project, Science and Technology Innovation Committee, Shenzhen, China.

REVIWER EXPERIENCES

1. Theranostics, 2020.01, manuscript 41006f1 (Targeting DNA repair in KRAS mutant lung cancer by chemical screen, Danmei Tian, Jinshan Tang, Xinran Geng, Jingwen Li, Fangfang Wang, Huadong Zhao, Mohamed Abazeed, Goutham Narla, Xinsheng Yao, and Youwei Zhang).

CONFERENCES and SEMINARS ATTENDED

June 22-24, 2020	Poster	AACR 2020 annual meeting, America (e-meeting)
October 24, 2019	Speaker Poster	The 14th International Conference on Genomics (ICG-14), Shenzhen, China
October 19-23, 2019	Poster	17th International Congress of Immunology, 17th ICI, IUIS 2019, Beijing, China
March 29-April 3, 2019	Participant	AACR Annual Meeting 2019, Atlanta, USA
April 26, 2018	Participant	MERK Novel Therapy, Shenzhen, China
March 30-31, 2018	Participant	Genome editing Conference, Beijing, China
December 1, 2017	Participant	South China Immunotherapy Clinical Research Forum, Shenzhen, China
November 17, 2017	Participant	Advanced Cell Therapy, Shanghai Summit 2017, Shanghai, China
October 22-26, 2017	Participant	Tumor Immunology＆Immunotherapy, Cold Spring Harbor Asia, Suzhou, China