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regulate the expression of Arpin. In multivariate models, the patients displaying an upregulation of the activator or a downregulation of the inhibitor have poor prognosis of metastasis-free survival [3]. Recently we analysed the combinatorial complexity of assembling WAVE and Arp2/3 complexes, because many subunits of these complexes are encoded by paralogous genes. The genes that were the most significantly associated with metastasis-free survival of breast cancer patients were also the most efficient at regulating cell migration. Statistical models of retrospective cohorts were even an efficient way to discover specific paralogous genes encoding subunits conferring singular behaviors to the complexes thatthey assemble.

Keywords: actin polymerization, Arp2/3 complex, nucleation promoting factors, molecular machines.

REFERENCES

1. *Molinie N, Gautreau A.* The Arp2/3 Regulatory System and Its Deregulation in Cancer. Physiol Rev. 2018; 98: 215–38.
2. *Dang I., Gorelik R., Sousa-Blin C., Derivery E., Guérin C., Linkner J., Nemethova M., Dumortier J.G., Giger F.A., Chipysheva T.A., Ermilova V.D., Vacher S., Campanacci V., Herrada I., Planson A.G., Fetics S., Henriot V., David V., Oguievetskaia K., Lakisic G., Pierre F., Steffen A., Boyreau A., Peyriéras N., Rottner K., Zinn-Justin S., Cherfils J., Bièche I., Alexandrova A.Y., David N.B., Small J.V., Faix J., Blanchoin L., Gautreau A.* Inhibitory signalling to the Arp2/3 complex steers cell migration. Nature. 2013 Nov 14; 503(7475): 281-4. doi: 10.1038/nature12611.
3. *Lomakina M.E., Lallemand F., Vacher S., Molinie N., Dang I., Cacheux W., Chipysheva T.A., Ermilova V.D., de Koning L., Dubois T., Bièche I., Alexandrova A.Y., Gautreau A.* Arpin downregulation in breast cancer is associated with poor prognosis. Br J Cancer. 2016 Mar 1; 114(5): 545-53. doi: 10.1038/bjc.2016.18.

RNA SEQUENCING OF SINGLE CELLS OBTAINED FROM IMMUNOLABELED TUMOR SECTIONS: THE FIRST EXPERIENCE

T.S. Gerashchenko[1,2], E.V. Denisov[1,2], A.M. Kiselev[3], N.M. Novikov[1,2], L.A. Tashireva[1], D.V. Pyankov[4], N.V. Cherdyntseva[1,2], V.M. Perelmuter[1]

Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[1]
Tomsk State University, Tomsk, Russia[2]
Almazov National Medical Research Centre, Saint-Petersburg, Russia[3]
Genomed Ltd, Moscow, Russia[4]

We performed laser microdissection-assisted RNA sequencing of single cells of breast tumor sections immunostained according the homemade protocol of maintaining RNA integrity.

Keywords: single-cell RNA sequencing, immunofluorescence, tumor heterogeneity.

Introduction

RNA sequencing (RNA-seq) is a highly informative method for characterizing transcriptional activity in different tissues and for studying molecular interactions between cells [1]. In cancer research, tumor complexity and heterogeneity challenge RNA-seq of tumor bulk that provides information regarding the average transcriptome and necessitate RNA-seq from single cells [2]. To date, single-cell RNA-seq became a powerful tool to profile cell-to-cell variability on a genomic scale [3]. This approach is critically important for the identification of tumor cell populations that are involved in cancer progression and sensitivity to treatment and of transcripts/genes associating with the realization of the metastatic cascade [2, 4]. In particular, the recent studies showed that single-cell transcriptome profiling can identify and characterize clinically important subpopulations of tumor cells to develop successful targeted treatments [5-7].

At present, single-cell RNA-seq is successfully applied to analyze transcriptomes of tumor cells obtained from hematoxylin and eosin stained sections using laser microdissection or from tumor bulk using fluorescence-activated cell sorting (FACS) [8, 9]. However, there are no any data regarding RNA-seq of tumor cells obtained from immunolabeled sections using laser microdissection. It is probable related to the problems of isolation of high-quality RNA samples from immunostained sections. Nevertheless, this approach would allow to assess transcriptome of tumor cells located in different geographic and microenvironmental regions of the tumor and to prevent the isolation of stromal and inflammatory cells that share tumor markers in FASC analysis.

In this study, we share the experience of RNA sequencing of single cells obtained from immunostained sections of frozen breast tumor samples using laser microdissection.

Method and Results

Frozen breast tumor samples were cryosectioned and used for immunofluorescence staining with antibodies to CK7 (sc-23876, 1:50, Santa Cruz), KIF14 (HPA038061, 1:500, Sigma), and WAVE-2 (1:500, [10]) proteins. We modified the protocol of immunostaining to avoid RNA degradation. In particular, we reduced time of incubation with antibodies and used PBS buffer with RNAlater (Thermo Fisher Scientific). Paired tumor sections were immunostained. First of them was used to estimate quality of RNA samples isolated using RNeasy Plus Micro Kit (Qiagen). RNA integrity number (RIN) varied from 5.6 to 6.3 (2200 Tape Station, Agilent). Second sections were used to isolate CK7+KIF14+, CK7+KIF14−, CK7+WAVE-2+ and CK7+WAVE-2− single cells (the number varied from 1 to 50) using laser microdissection guided under fluorescence (PALM, Carl Zeiss). Microdissected samples were used to generate cDNA libraries (SMARTer Stranded Total RNA-Seq Kit v2, Takara, USA). The quality of cDNA libraries was assessed using HS D1000 ScreenTape (2200 Tape Station, Agilent), the concentration – dsDNA HS kit (Qubit 4.0, Thermo Fisher Scientific). Library concentration varied from 0.694 to 92.2 ng/ul, fragment size ranged from 200 to 800 bp (average size of 359 bp). Libraries were pooled according Illumina recommendations and sequenced as single-end 75 bp reads on a NextSeq500 (Illumina). The total number of reads was ~450 mln for 19 samples pooled. At present, bioinformatic processing is performing; the detailed information will be presented in the presentation.

Conclusion

We developed and implemented the protocol of RNA sequencing of single cells obtained from immunolabeled tumor sections using laser microdissection. This approach represents an effective tool for the assessment of transcriptome of different tumor cell populations, especially in context of spatial intratumor heterogeneity.

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REFERENCES

1. *Costa-Silva J., Domingues D., Lopes F.M.* RNA-Seq differential expression analysis: An extended review and a software tool. PLOS One. 2017; 12(12): e0190152. doi:10.1371/journal.pone.0190152.

2. *Wang Y., Navin E.N.* Advances and applications of single cell sequencing technologies. Mol Cell. 2015 May 21; 58(4): 598-609. doi: 10.1016/j.molcel.2015.05.005.

3. *Saliba A-E., Westermann A.J., Gorski S.A., Vogel J.* Single-cell RNA-seq: advances and future challenges. Nucleic Acids Res. 2014 Aug; 42(14): 8845-60. doi: 10.1093/nar/gku555.

4. *Wang Z., Gerstein M., Snyder M.* RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009 Jan; 10(1): 57-63. doi: 10.1038/nrg2484.

5. *Chung W., Eum H.H., Lee H.O., Lee K.M., Lee H.B., Kim K.T., Ryu H.S., Kim S., Lee J.E., Park Y.H., Kan Z., Han W., Park W.Y.* Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. Nat Commun. 2017 May 5; 8: 15081. doi: 10.1038/ncomms15081.

6. *Miyamoto D.T., Zheng Y., Wittner B.S., Lee R.J., Zhu H., Broderick K.T., Desai R., Fox D.B., Brannigan B.W., Trautwein J., Arora K.S., Desai N., Dahl D.M., Sequist L.V., Smith M.R., Kapur R., Wu C.L., Shioda T., Ramaswamy S., Ting D.T., Toner M., Maheswaran S., Haber D.A.* RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science. 2015 Sep 18; 349(6254): 1351-6. doi: 10.1126/science.aab0917.

7. *Tirosh I., Izar B., Prakadan S.M., Wadsworth M.H., Treacy D., Trombetta J.J., Rotem A., Rodman C., Lian C., Murphy G., Fallahi-Sichani M., Dutton-Regester K., Lin J.R., Cohen O., Shah P., Lu D., Genshaft A.S., Hughes T.K., Ziegler C.G., Kazer S.W., Gaillard A., Kolb K.E., Villani A.C., Johannessen C.M., Andreev A.Y., Van Allen E.M., Bertagnolli M., Sorger P.K., Sullivan R.J., Flaherty K.T., Frederick D.T., Jané-Valbuena J., Yoon C.H., Rozenblatt-Rosen O., Shalek A.K., Regev A., Garraway L.A.* Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science. 2016 Apr 8; 352(6282): 189-96. doi: 10.1126/science.aad0501.

8. *Ståhl P.L., Salmén F., Vickovic S., Lundmark A., Navarro J.F., Magnusson J., Giacomello S., Asp M., Westholm J.O., Huss M., Mollbrink A., Linnarsson S., Codeluppi S., Borg Å., Pontén F., Costea P., Sahlén P., Mulder J., Bergmann O., Lundeberg J., Frisén J.* Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science. 2016 Jul 1; 353(6294): 78-82. doi: 10.1126/science.aaf2403.

9. *Villani A.C., Satija R., Reynolds G., Sarkizova S., Shekhar K., Fletcher J., Griesbeck M., Butler A., Zheng S., Lazo S., Jardine L., Dixon D., Stephenson E., Nilsson E., Grundberg I., McDonald D., Filby A., Li W., De Jager P.L., Rozenblatt-Rosen O., Lane A.A., Haniffa M., Regev A., Hacohen N.* Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science. 2017 Apr 21; 356(6335). pii: eaah4573. doi: 10.1126/science.aah4573.

10. *Gautreau A., Ho H-Y H., Li J., Steen H., Gygi S.P., Kikschner M.W.* Purification and architecture of the ubiquitous Wave complex. Proc Natl Acad Sci. U S A. 2004 Mar 30; 101(13): 4379–4383. doi: 10.1073/pnas.0400628101.