Evolution and a promising role of EUS-FNA in gene and future analyses

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Gene analysis with EUS-FNA was firstly reported in 2001, which revealed the diagnosis of gastrointestinal stromal tumors using mutational analysis of c-kit.[1] Since the first report published, there have been 121 English reports regarding genetic analysis with EUS-FNA samples except for review articles [Figure 1]. The most common site of the original lesions is the pancreas (64.5%) followed by the lung (25.6%) and so on. The sampled specimens by EUS-FNA from pancreatic diseases were mainly aspirates including cells and tissues from solid lesions and cyst fluid from cystic lesions, partially smears on the slide glass from solid lesions.

The initial gene analyses included a single gene mutation such as c-kit or K-ras, and gene expression of human telomerase reverse transcriptase with EUS-FNA samples (cells and tissues) from the gastric submucosal tumor, pancreatic cancer, and mediastinal metastatic lymph node of lung cancer using polymerase chain reaction (PCR), respectively.[1-3] Those results were dedicated to diagnoses. As technology such as DNA array was developed, the number of analyzed genes was increased up to some hundred genes in 2005.[4] Furthermore, in association with the progression of gene analysis in surgical specimens of lung cancer and pancreatic cancer, some gene mutations/fusions related to therapies were also detected in EUS-FNA specimens: KRAS, EGFR, and BRAF gene mutations related to the sensitivity of epidermal growth factor receptor inhibitors such as gefitinib/erlotinib and B-Raf inhibitors such as vemurafenib/ALK and ROS1 gene fusions related to the sensitivity of ALK inhibitor such as crizotinib in lung cancer; gemcitabine sensitivity-related mRNA (deoxycytidine kinase, human equilibrative nucleoside transporter 1 (hENT1), hENT2,

Figure 1. The number of published English reports regarding genetic analysis with EUS-FNA samples except for review articles according to each year. The columns are colored according to the site and number of the original lesion as shown in the color legend

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dCMP deaminase, cytidine deaminase, 5'-nucleotidase, ribonucleotide reductase 1 (RRM1), RRM2, and Notch 3) expressions in pancreatic cancer.\textsuperscript{[5,9]} Those outcomes were so-called beginnings of personalized medicine. However, the amount of specimen obtained by EUS-FNA was not generally sufficient for enormous genes, and comprehensive and genome-wide gene analysis was costly and time-consuming.

In 2004, the initial next-generation sequencing (NGS) technology (Roche/454 FLX Pyrosequencer: http://www. 454.com/enablingtechnology/the-system. asp) became available in the market and a trigger to overcome the problem of time and cost.\textsuperscript{[10]} In 2013, the result of NGS (454 GS-Junior) of multiple KRAS mutations and five distinct cell populations with a EUS-FNA specimen from one patient with pancreatic cancer was reported for the first time.\textsuperscript{[11]} Thereafter, 31 reports regarding NGS with EUS-FNA samples followed it, many of which used gene panels with several dozen– several hundred cancer-related genes. In solid pancreatic lesions, de Biase et al. demonstrated higher sensitivity up to 74% of KRAS mutations in pancreatic FNA specimens using NGS than that by allele-specific real-time PCR, maintaining specificity at 100%, while Gleeson et al. reported an excellent concordance between mutations (KRAS, TP53, SMAD4, and GNAS) detected in EUS-FNA specimens and those in the paired surgical materials: in 15 of 18 cases, the concordance was 100%.\textsuperscript{[12,13]} In cystic pancreatic lesions, NGS of cyst fluid is highly helpful to differentiate intraductal papillary mucinous neoplasm (IPMN) from other cystic pancreatic lesions with the combination of GNAs and KRAS testing. Jones et al. revealed that in 71% of the 92 samples, a KRAS or GNAS mutation was consistent with a diagnosis of IPMN by imaging, in spite of low carcinoembryonic antigen levels.\textsuperscript{[14]}

In 2011, 2016, and 2017, to solve the problem regarding sample acquirement and quantity, three new-type needles dedicated for EUS-guided fine-needle biopsy (FNB), a needle with reverse bevel, fork-tip needle, and Franseen needle, had been developed.\textsuperscript{[15]} Those needles have enabled us to more easily obtain sufficient tissue, regardless of the puncture site, compared with a conventional rigid biopsy needle. Asokkumar et al. indicated that the 22G Franseen EUS-FNB needle provides more histological core tissue (5.2mm$^2$ vs. 1.9mm$^2$, $P < 0.001$) and adequate nucleic acid yield (4,085ng vs. 2912ng, $P = 0.02$) compared to the 22G standard EUS-FNA needle. However, the diagnostic performance was similar between the needles.\textsuperscript{[16]} The novel needles should also make it possible to analyze microsatellite instability status in the specimen related to the effect of an immune checkpoint inhibitor in addition to driver gene mutations.

The difficulty of accurate NGS with EUS-FNA specimens also relies on the process of specimen treatment. First, the specimen needs to be treated to contain as high rate ($\geq$30%–50%) of tumor cells as possible for accurate analysis. The amount of DNA necessary for NGS depends on the platform, gene panel size, and target enrichment process. In the case of testing for hotspots in fifty genes with the multiplex PCR based Ion AmpliSeq™ Cancer Hotspot Panel (Life Technologies) on the Ion Torrent PGM requires 10 ng of input DNA, which accounts for 2000 target cells.\textsuperscript{[17]} Second, DNA in the specimen needs to be appropriately preserved for the analysis. For that, the specimen should be immediately fixed in 10% neutral buffered formalin for 6–48 h and embedded in paraffin as a block within 3 years.\textsuperscript{[18–20]} As another method for them, we acquired a specimen near the rapid on-site evaluation site and stored that in RNAlater (Life Technologies, Carlsbad, CA) at 4°C immediately after confirmation of malignancy during EUS-FNA of biliary tract cancer. As a result, pathogenic gene alterations were successfully identified in 20 out of 21 patients (95.2%) using NGS.\textsuperscript{[21]}

Analysis of genes is already essential in clinical practice and analysis of other new targets such as exosome, which carry diverse materials such as RNA, DNA, and unidentified molecules,\textsuperscript{[22]} with EUS-FNA samples must be necessary and promising for the refined diagnosis and precision medicine in the near future. Therefore, every endosonographer must get in touch with basic scientists and prepare for the newest technology.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Rader AE, Avery A, Wait CL, et al. Fine-needle aspiration biopsy diagnosis of gastrointestinal stromal tumors using morphology, immunocytochemistry, and mutational analysis of c-kit. *Cancer* 2001;93:269-75.

2. Tada M, Komatsu Y, Kawabe T, et al. Quantitative analysis of K-ras gene mutation in pancreatic tissue obtained by endoscopic ultrasonography-guided fine needle aspiration: Clinical utility for diagnosis of pancreatic tumor. *Am J Gastroenterol* 2002;97:2263-70.
3. Wallace MB, Block M, Hoffman BJ, et al. Detection of telomerase expression in mediastinal lymph nodes of patients with lung cancer. Am J Respir Crit Care Med 2003;167:1670-5.
4. Buchholz M, Kestler HA, Bauer A, et al. Specialized DNA arrays for the differentiation of pancreatic tumors. Clin Cancer Res 2005;11:8048-54.
5. van Eijk R, Licht J, Schrumpf M, et al. Rapid KRAS, EGFR, BRAF and PIK3CA mutation analysis of fine needle aspirates from non-small-cell lung cancer using allele-specific qPCR. PLoS One 2011;6:e17791.
6. Jurado J, Saqi A, Maxfield R, et al. The efficacy of EBUS-guided transbronchial needle aspiration for molecular testing in lung adenocarcinoma. Ann Thorac Surg 2013;96:1196-202.
7. Kirita K, Izumo T, Matsumoto Y, et al. Bronchoscopic re-biopsy for mutational analysis of non-small cell lung cancer. Lung 2016;194:371-8.
8. Ashida R, Nakata B, Shigekawa M, et al. Gemcitabine sensitivity-related mRNA expression in endoscopic ultrasound-guided fine-needle aspiration biopsy of unresectable pancreatic cancer. J Exp Clin Cancer Res 2009;28:83.
9. Eto K, Kawakami H, Kuwatani M, et al. Human equilibrative nucleoside transporter 1 and Notch3 can predict gemcitabine effects in patients with unresectable pancreatic cancer. Br J Cancer 2013;108:1488-94.
10. Mardis ER. Next-generation DNA sequencing methods. Annu Rev Genomics Hum Genet 2008;9:387-402.
11. Visani M, de Biase D, Baccarin P, et al. Multiple KRAS mutations in pancreatic adenocarcinoma: Molecular features of neoplastic clones indicate the selection of divergent populations of tumor cells. Int J Surg Pathol 2013;21:546-52.
12. de Biase D, Visani M, Acquaviva G, et al. The role of next-generation sequencing in the cytologic diagnosis of pancreatic lesions. Arch Pathol Lab Med 2018;142:458-64.
13. Gleeson FC, Kerr SE, Kipp BR, et al. Targeted next generation sequencing of endoscopic ultrasound acquired cytology from ampullary and pancreatic adenocarcinoma has the potential to aid patient stratification for optimal therapy selection. Oncotarget 2016;7:54526-54536.
14. Jones M, Zheng Z, Wang J, et al. Impact of next-generation sequencing on the clinical diagnosis of pancreatic cysts. Gastrointest Endosc 2016;83:140-8.
15. Cazacu IM, Luzziuriaga Chavez AA, Saftoiu A, et al. A quarter century of EUS-FNA: Progress, milestones, and future directions. Endosc Ultrasound 2018;7:141-60.
16. Asokkumar R, Yung Ka C, Loh T, et al. Comparison of tissue and molecular yield between fine-needle biopsy (FNB) and fine-needle aspiration (FNA): A randomized study. Endosc Int Open 2019;7:E955-63.
17. Chen H, Luthra R, Goswami RS, et al. Analysis of pre-analytic factors affecting the success of clinical next-generation sequencing of solid organ malignancies. Cancers (Basel) 2015;7:1699-715.
18. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784-95.
19. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013;8:823-59.
20. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: A joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn 2017;19:341-65.
21. Hirata K, Kuwatani M, Suda G, et al. A novel approach for the genetic analysis of biliary tract cancer specimens obtained through endoscopic ultrasound-guided fine needle aspiration using targeted amplicon sequencing. Clin Transl Gastroenterol 2019;10:e00022.
22. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 2020;367. pii: eaa46977.