

Translational research in thoracic surgery—the National Taiwan University Hospital experience

Mong-Wei Lin, Pei-Wen Yang, Jang-Ming Lee

Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Contributions: (I) Conception and design: JM Lee; (II) Administrative support: JM Lee; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Jang-Ming Lee, MD, PhD. Division of Thoracic Surgery, Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, No. 7, Chung-Shan South Road, Taipei 10002, Taiwan. Email: jmlee@ntu.edu.tw.

Abstract: Thoracic surgeons should be more aware of the latest information about histopathological, genetic and epigenetic alterations that may influence treatment policy and patient outcome in the biomolecular era. Translational research studies often produce a promising diagnostic tool or new treatment that can be used clinically. The results of these translational studies may even change the practical guidelines and current staging system in thoracic malignancies. The following article summarizes the experiences of translational research in esophageal cancer and non-small cell lung cancer (NSCLC) at National Taiwan University Hospital in Taiwan.

Keywords: Translational research; esophageal cancer; non-small cell lung cancer (NSCLC); thoracic surgery

Submitted Jun 08, 2016. Accepted for publication Jun 16, 2016.

doi: 10.21037/jtd.2016.07.95

View this article at: <http://dx.doi.org/10.21037/jtd.2016.07.95>

Introduction

The treatment of thoracic malignancies has become a multidisciplinary approach that incorporates surgery, chemotherapy, radiotherapy, targeted therapy, immunotherapy, photodynamic therapy (PDT), and other treatment modalities. Therefore, thoracic surgeons should be more aware of the latest information about the histopathological, genetic and epigenetic alterations that may influence treatment policy and patient outcome in the biomolecular era. It is no longer practical for thoracic surgeons to ignore the biomolecular information that may affect our diagnosis and treatment, such as mutations with target therapies and biomarkers related to immunotherapies. Thoracic surgeon-scientists have to pay more attention to the biomolecular translational studies that may be related to the clinical outcomes and treatment policies, from ward to operating room.

What exactly is translational research? According to the definition of the European Society for Translational

Medicine, translational research is an interdisciplinary branch of the biomedical field supported by three main pillars: bench-side, bedside and community research (1). The goal of translational medicine is to combine disciplines, resources, expertise, and techniques within these pillars to promote improvements in prevention, diagnosis, and therapy (1). The goal of translational research is usually to produce a promising diagnostic tool or new treatment that can be used clinically. Many thoracic surgical societies have perceived the increasing relevance of biomolecular translational research in the practice of modern thoracic surgery (2). This translational research may even change the practical guidelines and current staging system used in treating thoracic malignancies.

It is not easy for a thoracic surgeon-scientist to do excellent work in the operating room, in the intensive care unit, in the ward, and in the laboratory. The clinical workload is usually high for thoracic surgeons in Taiwan. There are more than 2,500 operations performed per year in the thoracic surgery division of National Taiwan

University Hospital, and there are only seven attending physicians in our division. Therefore, it is important to cooperate with experts in other fields to do translational research. The experts should include pathologists, molecular biologists, statisticians and bioinformatics specialists. In the following manuscript, we summarize our experience with translational research in esophageal cancer and non-small cell lung cancer (NSCLC) in the National Taiwan University Hospital.

Esophageal cancer

Esophageal cancer is one of the major causes of cancer death worldwide. Primary esophageal cancer presents most often as esophageal squamous cell carcinoma (ESCC) or adenocarcinoma (EAC) (3-5). ESCC is the major cell type of primary esophageal cancer accounting for 95% of the disease and prevalent in non-Caucasian populations, particularly in southern Africa and some Asian regions, including in Taiwan (4,5).

The standard management for locally advanced esophageal cancer is preoperative concurrent chemoradiotherapy (CCRT) and surgery. Patients with good response to CCRT usually have better clinical outcome. However, the pathological complete remission rate is usually less than 40% (6-8). The prognosis of esophageal cancer is relative poor, with a reported 5-year survival rate usually less than 20% (3,4,9). To predict the prognosis and develop biomarker-based personalized therapy, we have searched for prognostic biomarkers of esophageal cancer. We have focused on the study of the prognostic relevance of germline single nucleotide polymorphisms (SNPs) involved in cancer-related pathways, including nucleotide excision repair (NER), micro-RNA (miRNA) processing, and receptor tyrosine kinase (RTK)/growth factor signaling pathways. In a study of NER-related SNPs, we found that the C/C genotype of ERCC2_R156R and the C/T genotype of ERCC4_rs3136038 cumulatively increase risk of mortality and disease progression of esophageal cancer patients (N=400) (10). A total of 549 patients with esophageal cancer were enrolled in another of our studies to analyze the genetic effects of XPA in 5'UTR and XPC at exon 15 K939Q. The genetic variants of XPA_A23G A/G and XPC_K939Q C/C were found to be correlated with both unfavorable overall and progression-free survival, and XPC_K939Q C/C was also significantly correlated with poor-response to CCRT (11).

The genetic polymorphisms of miRNA, miRNA-targeted,

and miRNA-related genes have also been demonstrated to significantly correlate with varied clinical outcome of several cancers. We have demonstrated that the SNP rs4919510 contained in the mature sequence of miR-608 microRNA can predict the prognosis of ESCC. Both the homozygous CC and GG genotypes showed unfavorable prognosis compared to the CG genotype. Meanwhile, the TT genotype of RAN and the TC genotype of GEMIN4 displayed adverse effects on overall survival. Patients carrying all 3 unfavorable genotypes exhibited a 2.60-fold risk for mortality compared to patients without any of the adverse genotypes (12).

Growing reports reveal a significant association between the prognosis of malignancy and both expression and occurrence of SNPs involved in the RTK/growth factor signaling pathway. We reported that a polymorphism in the epidermal growth factor receptor (EGFR) intron 1, the CA repeat polymorphism, predicted the prognosis of our patients with esophageal cancer after CCRT and surgery. We showed that those who with the L/L (L: long) genotype had a significantly lower hazard ratio (HR) of death as compared to patients with the EGFR S/S (S: short) genotype (13). We followed up that result by systematically searching for prognostically-relevant SNPs involved in RTK/growth factor functions in 334 patients with advanced ESCC. The SNPs of EGF:rs4444903, EGF:rs2237051 and VEGF:rs2010963 showed significant associations with the overall survival of advanced ESCC patients. EGFR:rs2227983 and three SNPs of PIK3CA also displayed borderline significant association with survival of advanced ESCC (14). Cumulative analysis revealed patients carrying four unfavorable genotypes exhibited an increased risk of death by more than 3-fold (14).

ESCC is a difficult thoracic disease; however, there is no clinically approved targeted drug for this deadly cancer. Searching for effective biomarker-based targeted therapies is an emergent issue in ESCC treatment. Many drugs targeting RTKs have been evaluated in ESCC. The small molecular inhibitors for EGFR, gefitinib (Iressa) and erlotinib (Tarceva) have been evaluated in esophageal cancer in phase II and phase III studies (15-18). Disease control after second-line gefitinib treatment was observed in advanced ESCC patients in a phase II study (5 of 9 patients; 55 %) (16). However, in a large-sized phase III study, the use of gefitinib as a second-line treatment in esophageal cancer did not result in improvement of overall survival (15). Response to erlotinib was also observed in patients with ESCC in a phase II study (18). In addition to targeting EGFR, other targeting agents

have been studied for treatment of ESCC, such as drugs targeting HER2/Neu, the mammalian target of rapamycin (mTOR), and vascular endothelial growth factor (VEGF), though most have been restricted to pre-clinical tests or phase I studies (19).

In our study, serum VEGF and EGF (epidermal growth factor) levels, but not tumor EGFR expression, were associated with mortality of patients with advanced ESCC (14). We thus suggested a therapeutic approach for treating local advanced ESCC by considering serum levels of EGF and VEGF as biomarkers. AXL RTK has demonstrated potential to become a novel target for cancer targeted therapy, including in EAC (20). We recently demonstrated that patients positive for AXL in tumor tissue have increased risks of death and distant metastasis, and their median survival time dramatically decreased from about 47 to 14 months. We also found that the unfavorable clinical effect of AXL was more marked when cumulatively expressed with HER2 RTK (21). In the cell model, ESCC cells were relatively sensitive to the AXL inhibitor foretinib compared to HER2 inhibitors and that foretinib exhibited a synergistic effect with HER2 inhibitors (21). Therefore, we suggested therapeutic drugs targeting AXL as a potential approach to developing targeted therapy for ESCC.

PDT is a minimally invasive treatment used for many malignancies, and has been demonstrated as an effective procedure for early esophageal cancer (22-25). In the literature, the complete remission rate of the Photofrin-based PDT (Photofrin-PDT) for early esophageal cancer is around 70% and 5-year survival around 60% according to the reports published in the last two decades, rates comparable to those obtained with surgery (23,26,27). Since the molecular mechanism of the resistance to PDT in esophageal cancer cells has hardly been investigated, an effective molecular marker available to predict response to PDT is lacking. We have demonstrated that Photofrin modulates expression of EGFR in ESCC cells. Combination of light irradiation with Photofrin is still required for efficient cell death in ESCC cells (28).

In order to explore possible cellular factors involved in resistance to Photofrin-PDT in ESCC cells, we analyzed the global gene expression changes in PDT-resistant cells by whole-genome microarray. We found that both EFNA1 and TNF (tumor necrosis factor) genes were up-regulated in resistant cells and down-regulated in Photofrin-PDT-treated cells relative to untreated cells (29). EFNA1 encodes ephrin-A1, which belongs to a member of the EPH (ephrin)

family, the largest subfamily of the RTKs, and plays a crucial role in tumor growth, metastasis and angiogenesis (30). We demonstrated that TNF- α (a protein product of TNF) stimulated the gene expression of EFNA1 in ESCC cells. Functional analysis revealed ESCC cells became obviously resistant to Photofrin-PDT when incubated with oligomeric and monomeric ephrin-A1 simultaneously. Therefore, we suggested that soluble and transmembrane ephrin-A1 might cooperate to enhance resistance to Photofrin-PDT in ESCC cells (29).

NSCLC

Because of the prevalence of low dose chest computed tomography screening, multiple primary lung cancers (MPLCs) are detected more frequently than in the past (31). However, the genetic profile, treatment, and prognosis of patients remain unclear. The current criteria to differentiate MPLCs from metastases are based on histologic type and onset interval and do not incorporate genetic analysis (32). The genetic background of MPLCs remains unclear. Therefore, we investigated the EGFR/p53/KRAS genetic profiles of both metachronous and synchronous MPLCs (33,34). Our findings further supported the carcinogenic theory of field cancerization. Because most multiple lung tumors of the same histologic type have different clonal origins, we suggested that clonality assessment is essential to differentiate MPLCs from metastases.

Programmed death ligand 1 (PD-L1)-mediated immune escape may be an underlying source of resistance and a suitable target for specific therapy, but its role in early stage NSCLCs is unclear. We investigated the prognostic roles of PD-L1 and the immune microenvironment in different types of early stage NSCLC, including adenocarcinoma (35), squamous cell carcinoma (36), lung lymphoepithelioma-like carcinoma (LELC) (37) and pleomorphic carcinoma (38). PD-L1 expression can be used as a prognostic indicator predictive of better survival in patients with surgically resected stage I lung adenocarcinomas and squamous cell carcinoma (35,36). High PD-L1 expression and infrequent driver mutation was found in LELCs compared with conventional NSCLCs (37). The high expression of PD-L1 in inflammation associated LELC may provide a rationale for immunotherapy in this subtype of lung cancer. High PD-L1 and HIF-1 α co-expression was observed in pleomorphic carcinomas compared with their expression in conventional NSCLCs (38). The aggressive behavior of

pleomorphic carcinoma could be partially related to PD-L1-mediated immune escape and intratumoral hypoxia. High PD-L1 expression correlates with poor prognosis and may provide a rationale for the use of targeted immunotherapy in this subtype of high-grade pleomorphic carcinoma (38).

Tumor-associated macrophages (TAMs) play an important role in the initiation, progression, and metastasis of various solid tumors, and can polarize into M1 and M2 phenotypes (39). We investigated whether TAM polarization was associated with clinical outcomes for early-stage pulmonary squamous cell carcinoma (40). Multivariate analysis showed that the pSTAT1 (M1 marker)/CD163 (M2 marker) expression status was the only independent predictor for both disease-free survival ($P=0.023$) and overall survival ($P=0.004$). Markers identifying M1 and M2 macrophages, including pSTAT1 and CD163, can thus be used as prognostic indicators for patients with stage 1 pulmonary squamous cell carcinoma (40).

Conclusions and further perspectives

We have summarized recent results of translational research in esophageal cancer and NSCLCs in our institute. Most of the results can be translated into the practice of thoracic surgeons and applied in the clinical treatment of thoracic malignancies. In addition to translational research, prospective, multicenter clinical trials can provide more reliable evidence that these findings of translational research indeed improve clinical practice and thus the outcome of patients with thoracic malignancies. Further collaboration among multiple medical centers in prospective clinical trials is important to allow Taiwanese thoracic surgeons to validate the evidence of new prognostic biomarkers as well as the treatment methods derived from translational research.

Acknowledgements

Funding: This study was supported in part by the Ministry of Science and Technology (MOST 104-2314-B-002-182-MY3), National Taiwan University Hospital (NTUH.105-S3005), Taiwan Health Foundation, and Taiwan Society for the Chest Care of Taiwan, ROC.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Lin MW, Yang PW, Lee JM. Translational research in thoracic surgery—the National Taiwan University Hospital experience. *J Thorac Dis* 2016;8(Suppl 8):S642-S647. doi: 10.21037/jtd.2016.07.95