

Molecular Pathology of Oesophageal Tumours

Author

Lam, Alfred

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MOLECULAR PATHOLOGY OF OESOPHAGEAL TUMOURS

By

Alfred King-Yin Lam

M.B.B.S., F.R.C.P.A.

School of Medicine, Faculty of Health, Griffith University

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the degree of Doctor of Philosophy

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ABSTRACT

Oesophageal tumours are very common worldwide. This thesis aims to delineate the clinicopathological features and molecular biology of oesophageal tumours in Hong Kong Chinese. Over a 30-year study period, oesophageal tumours were obtained in the pathology files of the Queen Mary Hospital, Hong Kong.

The tumours were prevalent in males and had a modal peak occurrence in the 7th decade. Patients often presented at an advanced stages. At autopsy, the prevalence of incidental oesophageal cancers and early-stage cancers were low.

Many histological subtypes of oesophageal cancers were noted and were different from the Western populations. The most common histological subtype was squamous cell carcinoma, often moderately-differentiated. Besides the classical squamous cell carcinomas, variants like mucoepidermoid carcinoma/adenosquamous carcinoma, basaloid squamous carcinoma and sarcomatoid carcinoma were noted. The prognosis of squamous cell carcinoma with a mucin-secreting component (mucoepidermoid carcinoma and adenosquamous carcinoma) was not significantly different from that of patients with pure squamous cell carcinoma or adenocarcinoma. The glandular component of this group of tumours histochemically differentiated in the direction of oesophageal glands. Basaloid squamous cell carcinoma had distinctive clinicopathological features and its long-term prognosis was no worse than squamous cell carcinoma. Glomerulonephritis could be a para-neoplastic manifestation of basaloid squamous carcinoma of oesophagus. Sarcomatoid carcinomas were also found, and rarely double sarcomatoid carcinomas could be noted in the same patient.

The other carcinomas noted in the oesophagus were small cell carcinoma and adenocarcinoma. Oesophageal small cell carcinoma was an aggressive tumour. The high proliferative index correlates with aggressive behaviour and high sensitivity to chemotherapy and radiotherapy. Oesophageal adenocarcinoma was uncommon in Hong Kong. On the other hand, intestinal metaplasia, known to be associated with adenocarcinoma, was prevalent at the gastroesophageal junction in Chinese patients undergoing endoscopy.

The non-epithelial tumours in the oesophagus comprised melanoma and mesenchymal tumours. Melanoma of the oesophagus was an aggressive tumour. All patients with the tumour had short survival. Mesenchymal tumours consisted of leiomyoma, undifferentiated stromal tumour and autonomic nerve tumour.

Intramural metastasis and multiple tumours were frequently observed in oesophageal cancer. This implies that wide excision with wide margins should be considered for local control of the disease. Pre-operative chemotherapy was commonly employed for the treatment of oesophageal cancer. High-grade nuclear pleomorphism in oesophageal carcinomas was correlated to chemo-responsiveness of the tumour.

Four cancer cell lines were established from patients with oesophageal squamous cell carcinomas. These newly established cell lines serve as a useful model for studying the molecular pathogenesis, and testing new therapeutic reagents for oesophageal squamous cell carcinoma.

Proliferative activity, as defined by the MIB-1 labelling index, was related to tumour differentiation in oesophageal squamous cell carcinoma. The activity was high in poorly-differentiated squamous cell carcinoma, basaloid squamous carcinoma and small

cell carcinoma of the oesophagus. MIB-1 labelling index was found to be valuable as an independent prognostic marker in addition to tumour stage and size. Image analysis could assist in counting of the proliferative activity.

Human papilloma virus was detected in a small proportion of oesophageal squamous cell carcinomas. There was no correlation between the prevalence of HPV and p53 mutation in these tumours. Epstein Barr virus was not detected in squamous cell carcinomas and mesenchymal tumours.

The pattern of expression of cytokeratins in oesophageal carcinomas is different from that in normal oesophageal epithelia and varies with differentiation.

Cancer-related genes studied in oesophageal cancers were p53, p21, c-erbB-2, PTEN and telomerase activity. p53 mutations were common in oesophageal squamous cell carcinomas and small cell carcinomas. The distribution of p53 mutations in oesophageal cancers suggests that the gene has complex exogenous and endogenous interactions. p53 mutations also appear to play a role in predicting the survival of patients with stage III oesophageal squamous cell carcinomas. The pattern of p21 and p53 expression predicts an aggressive clinical course of oesophageal squamous cell carcinomas. c-erbB-2 (Her-2) oncoprotein was expressed in a portion of oesophageal squamous cell carcinomas and precursor lesions. This suggests that c-erbB-2 activation plays a certain role, mostly probably during the early stages, in carcinogenesis. PTEN/MMAC1 mutations were not detected in oesophageal squamous cell carcinoma. Telomerase activation was common in small cell carcinoma and basaloid squamous cell carcinoma of the oesophagus. The level of telomerase activity had a prognostic role in

oesophageal cancer, suggesting a possible therapeutic role of anti-telomerase treatment for this aggressive tumour.

Multiple genetic mutations in oesophageal squamous cell carcinomas could be mapped by gene arrays and comparative genomic hybridization. These different newly discovered genetic alterations were analysed both in laboratory and in relationship with the clinical prognosis. Multiple mutations could be detected in dysplasia as well as carcinoma. The techniques identify the roles of some new cancer-related genes like Fra-1, Neogenin, Id-1, CDC25B and MET in oesophageal squamous cell carcinomas. Chromosomal aberrations were common in oesophageal squamous cell carcinoma. Gain in 12p was found to be indicative of aggressive behaviour and poor prognosis.

In summary, identification of the different histological types of oesophageal tumours and their characteristic molecular profiles is essential for both in-depth research and clinical management.

STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university.

To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.



Signed

Alfred King Yin Lam

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INTRODUCTION

A. Publications presented for examination

1. **Lam KY**, Law S, Wong J. Low prevalence of incidentally discovered and early-stage oesophageal cancers in a 30-year autopsy study. *Dis Esophagus* 2003;16:1-3.
2. **Lam KY**, Ma L. Pathology of oesophageal cancers: local experience and current insights. *Chin Med J* 1997;110:459-64.
3. Fegelman E, Law SYK, Fok M, **Lam KY**, Loke SL, Ma LT, Wong J. Squamous cell carcinoma of the esophagus with mucin-secreting component (mucoepidermoid carcinoma). *J Thoracic Cardiovasc Surg* 1994;107:62-67.
4. **Lam KY**, Loke SL, Ma LT. Histochemistry of the mucin-secreting components in mucoepidermoid and adenosquamous carcinoma of the esophagus. *J Clin Pathol* 1993;46:1011-15.
5. **Lam KY**, Law S, Tung PH, Wong J. Oesophageal basaloid squamous cell carcinoma: an unique clinicopathological entity with telomerase activity as a prognostic indicator. *J Pathol* 2001;195:435-42.
6. **Lam KY**, Law SYK, Chan KW, Yuen MC. Glomerulonephritis associated with basaloid squamous cell carcinoma of the esophagus. *Scand J Urol Nephrol* 1998;32:61-63.
7. **Lam KY**, Law SYK, Loke SL, Fok M, Ma LT. Double sarcomatoid carcinomas of the esophagus. *Path Res Pract* 1996;192:604-9.
8. **Lam KY**, Law S, Tung PH, Wong J. Esophageal small cell carcinoma: clinicopathologic parameters, p53 overexpression, proliferative marker, and their

- impact on pathogenesis. *Arch Pathol Lab Med* 2000;124:228-233.
9. Law S, **Lam KY**, Chu KM, Wong J. Specialized intestinal metaplasia and carditis at the gastroesophageal junction in Chinese patients. *Am J Gastroenterol*; 2002;97:1924-29.
 10. **Lam KY**, Law S, Wong J. malignant melanoma of the oesophagus: clinicopathological features, lack of p53 expression and steroid receptors and a review of the literature. *Eur J Surg Oncol* 1999;25:168-72.
 11. **Lam KY**. Oesophageal mesenchymal tumours: clinicopathological features and absence of Epstein-Barr virus. *J Clin Pathol* 1999;52:758-60.
 12. **Lam KY**, Law SYK, Chu KM, Ma LT. Gastrointestinal autonomic nerve tumour of the esophagus: a clinicopathologic, immunohistochemical, ultrastructural study of a case and review of the literature. *Cancer* 1996;78:1651-59.
 13. **Lam KY**, Ma LT, Wong J. Measurement of extent of spread of esophageal squamous carcinoma by serial sectioning. *J Clin Pathol* 1996;49:124-9.
 14. **Lam KY**, Law S, Ma LT, Ong SK, Wong J. Pre-operative chemotherapy for squamous cell carcinoma of the esophagus: do histological assessment and p53 overexpression predict chemo-responsiveness? *Eur J Cancer* 1997;33:1221-25.
 15. Hu YC, **Lam KY**, Wan TSK, Fang WG, Ma ESK, Chan LC, Srivastava G. Establishment and characterization of HKESC-1, a new cancer cell line from human oesophageal squamous cell carcinoma. *Cancer Genet Cytogenet* 2000;118:112-20.

16. Tang JCO, Wan TSK, Wong N, Pang E, **Lam KY**, Law SYK, Chow LMC, Ma ESK, Chan LC, Wong J, Srivastava G. Establishment and characterization of a new xenograft-derived human oesophageal squamous cell line SLMT-1 of Chinese origin. *Cancer Genet Cytogenet* 2001;124:36-41.
17. Hu YC, **Lam KY**, Law SYK, Wan TSK, Ma ESK, Kwong YL, Chan LC, Wong J, Srivastava G. Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human oesophageal squamous cell lines. *Cancer Genet Cytogenet* 2002;135:120-27.
18. **Lam KY**, Law SYK, So MKP, Fok M, Ma LT, Wong J. Prognostic implication of proliferative markers MIB-1 and PC 10 in oesophageal squamous cell carcinoma. *Cancer* 1996;77:7-13.
19. Law AKW, **Lam KY**, Lam FK, Wong TKW, Poon JLS, Chan FHY. Image analysis system for assessment of immunohistochemistry stained proliferative marker (MIB-1) in oesophageal squamous cell carcinoma. *Comp Methods Programs Biomed* 2003;70:37-45.
20. **Lam KY**, Ma L, Law SYK, Cheung LKN, Luk TF, Wong J. Use of flow cytometry in the analysis of stage III squamous cell carcinoma of the esophagus and its association with MIB-1. *J Clin Pathol* 1996;49:975-78.
21. **Lam KY**, He D, Ma L, Zhang D, Ngan HYS, Wan TSK, Tsao SW. Presence of human papillomavirus in esophageal squamous cell carcinomas of Hong Kong Chinese and its relationship with p53 gene mutation. *Hum Pathol* 1997;28:657-63.

22. **Lam KY**, Srivastava G, Leung ML, Ma L. Absence of Epstein-Barr virus in esophageal squamous cell carcinoma: a study of 74 cases using in-situ hybridization. *J Clin Pathol Mol Pathol* 1995;48:M188-90.
23. **Lam KY**, Tsao SW, Zhang D, Law S, He D, Ma L, Wong J. Prevalence and predictive value of p53 mutation in patients with oesophageal squamous cell carcinomas: a prospective clinicopathological study and survival analysis of 70 patients. *Int J Cancer* 1997;74:212-19.
24. **Lam KY**, Law S, Lo T, Tung HM, Wong J. The clinicopathological significance of p21 and p53 expression in oesophageal squamous cell carcinoma: an analysis of 153 patients. *Am J Gastroenterol*; 1999; 94: 2060-68.
25. **Lam KY**, Tin L, Ma L. c-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. *Eur J Surg Oncol* 1998;24:431-35.
26. Hu YC, **Lam KY**, Tang JCO, Srivastava G. Mutational analysis of the PTEN/MMAC1 gene in primary oesophageal squamous cell carcinomas. *J Clin Pathol Mol Pathol* 1999;52:353-56.
27. Chow V, Law S, **Lam KY**, Luk JM, Wong J. Telomerase activity in small cell oesophageal carcinoma. *Dis Esophagus* 2001;14:139-42.
28. **Lam KY**, Loke SL, Shen XC, Ma LT. Cytokeratin expression in non-neoplastic oesophageal epithelium and squamous cell carcinoma of the esophagus. *Virchows Arch A* 1995;426:345-49.

29. Tang JCO, **Lam KY**, Law S, Wong J, Srivastava G. Detection of genetic alterations in oesophageal squamous cell carcinomas and adjacent normal epithelia by comparative DNA fingerprinting using inter-simple sequence repeat PCR. *Clin Cancer Res* 2001;7:1539-45.
30. Hu YC, **Lam KY**, Law S, Wong J, Srivastava G. Identification of differentially expressed in oesophageal squamous cell carcinoma (ESCC) by cDNA expression array: overexpression of Fra-1, Neogenin, Id-1 and CDC25B genes in ESCC. *Clin Cancer Res* 2001;7:2213-21.
31. Hu YC, **Lam KY**, Law S, Wong J, Srivastava G. Profiling of differentially expressed cancer-related genes in oesophageal squamous cell carcinoma (ESCC) using human cancer cDNA arrays: overexpression of oncogene MET correlates with tumour differentiation in ESCC. *Clin Cancer Res* 2001;7:3519-25.
32. Kwong D, **Lam A**, Guan X, Law S, Tai A, Wong J, Sham J. Chromosomal aberrations in oesophageal squamous cell carcinoma among Chinese: gain of 12p predicts poor prognosis after surgery. *Hum Pathol* 2004;35:309-16.

B. The way in which the work, as manifest in the publications, has developed

Oesophageal tumour is a common disease; oesophageal cancer is a major cause of cancer death worldwide and is particularly common in certain areas in Asia, including Hong Kong. There are many histologic subtypes of oesophageal tumour. The proportion of various histologic subtypes in Hong Kong is different from the low incidence regions. Advances in deeper understanding of the process of carcinogenesis may allow prevention, diagnosis, and treatment of many cancers to be approached at the molecular level. However, compared with other cancers, little in-depth research has been performed in oesophageal cancers.

In view of this, the author performed studies with the objectives to increase understanding of the behaviour of oesophageal tumours and to improve the management of patients with these tumours. The topics included the clinicopathological status of the disease and the application of molecular biology in understanding pathogenesis and management of the tumours.

The submitted material consists of work done on the patients with oesophageal tumours diagnosed in Hong Kong Chinese in a 30-year study period. The studies were based on materials (surgical or autopsy) collected from more than a thousand of patients with esophageal cancers treated in a single institution over a 30-year study period. The pathology features, clinical data, survival data and molecular biology findings of these tumours and their relationships were analysed and presented in these publications. The first portion of the submitted materials deals with classification and clinicopathological features of oesophageal tumours. The findings provided reliable resources for the second portion of the submitted thesis that studied the impact of molecular biology on the understanding the pathogenesis and management of patients with oesophageal tumours.

C. Contemporary relevance of publications

The contemporary relevance of each publication was listed in the following:

1. Lam KY, Law S, Wong J. Low prevalence of incidentally discovered and early-stage oesophageal cancers in a 30-year autopsy study. *Dis Esophagus* 2003;**16**:1-3.

AIMS: Autopsy studies provide information that may guide future patient management.

The aims were to study the features of oesophageal cancers at autopsy.

METHOD: This study analyzed autopsy findings in patients with oesophageal cancers, with emphasis on the prevalence of incidentally diagnosed oesophageal cancer, histologic subtypes, early-stage lesions, and any associated pathology.

RESULTS: Autopsies detected 346 patients (306 men; 40 women) with oesophageal carcinomas during a 30-year period, constituting an overall prevalence of 3.4%. Out of these patients, 30 (8.7%) were incidentally discovered at autopsy. Squamous cell cancers were found in 336 (97.1%) patients, small cell cancers in nine (2.7%), and adenocarcinomas in one (0.3%). Stage distributions were stage I in seven patients (2%), stage II in 49 (14%), stage III in 121 (35%), and stage IV in 169 (49%). Isolated dysplasia or carcinoma-in-situ were not found. Comparing with symptomatic patients, patients with incidental cancers were older, had higher frequency of small cell carcinomas, and lower T-stage.

CONCLUSION: The prevalence of incidentally diagnosed oesophageal carcinomas and early-stage carcinomas were low. Unusual histologic subtypes may be found.

2. Lam KY, Ma L. Pathology of oesophageal cancers: local experience and current insights. *Chin Med J* 1997;**110**:459-64.

AIMS: Oesophageal cancer is very popular in Hong Kong. The tumour is often prevalent in males and has a modal peak of occurrence in the 7th decade. The aim is to study the pathology of the oesophageal cancer in Hong Kong.

METHOD: The prevalence of different histological subtypes and pathological features of oesophageal cancers were analysed.

RESULTS: The most common histological subtype is squamous cell carcinoma. Besides, mucoepidermoid carcinoma/adenosquamous carcinoma, adenocarcinoma, small cell carcinoma, sarcomatoid carcinoma, adenoid cystic carcinoma, undifferentiated carcinoma, melanoma and gastrointestinal autonomic nerve tumour are also found in a minority of patients.

CONCLUSION: Many histological subtypes of oesophageal cancers were noted. The proportion of various histologic subtypes is different from the Western populations.

3. Fegelman E, Law SYK, Fok M, **Lam KY**, Loke SL, Ma LT, Wong J. Squamous cell carcinoma of the oesophagus with mucin-secreting component (mucoepidermoid carcinoma). *J Thoracic Cardiovasc Surg* 1994;**107**:62-67.

AIMS: The aims are to study the clinicopathological features and outcomes of squamous cell carcinoma of the oesophagus with mucin-secreting component.

METHOD: Among 1058 patients with cancer of the oesophagus, 20 patients with mucoepidermoid or adenosquamous cell carcinoma of the oesophagus and cardia, together defined as squamous cell carcinoma with a mucin-secreting component, were seen over a 10-year period. Their records were reviewed and appropriate comparisons were also made with the more common squamous cell carcinomas and adenocarcinomas.

RESULTS: Squamous cell carcinoma with mucin-secreting component comprised 1.9% of all tumours encountered. Clinical features including age, male predominance, symptoms at presentation, length of tumour, and appearance of tumour did not differ from those of squamous cell and adenocarcinoma. The location of these tumours, however, followed that of squamous cell carcinomas, with 55% in the middle third and 25% in the lower third. Adenocarcinomas were found predominantly at the cardia (83%). Operability and resectability rates were higher than those of squamous cell and adenocarcinomas. Primary treatment consisted of resection in 19 of the 20 patients (95%); 18 of them had a one-stage resection and 1 patient had a two-stage resection. Post-resection staging showed that 5% had stage I disease, 16% had stage II, and 79% had stage III disease. None of the patients who underwent resection died within 30 days of the operation. The mortality after 30 days was 10.5%. The 1 patient in whom intubation was the primary treatment had distant metastases at the time of presentation

(stage IV). The overall median survival was 9.2 months. The median survival for patients who had their tumours resected was 9.5 months. The survival improved to 33 months for curative resection but was only 8.7 months for palliative resection. The 1-, 2-, and 5-year survivals were 46%, 39%, and 0%, respectively.

CONCLUSION: The prognosis of this carcinoma was not significantly different from that of patients with squamous cell carcinoma or adenocarcinoma.

4. Lam KY, Loke SL, Ma LT. Histochemistry of the mucin-secreting components in mucoepidermoid and adenosquamous carcinoma of the oesophagus. *J Clin Pathol* 1993;**46**:1011-15.

AIMS: To determine the direction of differentiation of the mucin secreting components in a rare group of oesophageal tumours--oesophageal squamous cell carcinomas with prominent mucin secreting components (mucoepidermoid carcinomas and adenosquamous carcinomas).

METHOD: In a review of 617 cases of primary carcinoma of the oesophagus, 16 cases of squamous cell carcinoma with prominent mucin secreting components were studied using a battery of histochemical techniques.

RESULTS: The mucin produced by these tumours was mixed and included a variable content of enzyme labile sialomucin (positive for mucicarmin, periodic acid Schiff, and alcian blue, and sensitive to sialidase digestion and negative for high iron diamine-alcian blue). Retrospective analysis of endoscopic biopsy specimens taken from these tumours showed that mucin was present in five (42%) cases.

CONCLUSION: The glandular component of this group of tumours histochemically differentiated in the direction of oesophageal glands: examination of the mucin secreting component in squamous cell carcinoma in resected specimens is therefore required for recording the true incidence of this type of tumour.

5. Lam KY, Law S, Tung PH, Wong J. Oesophageal basaloid squamous cell carcinoma: an unique clinicopathological entity with telomerase activity as a prognostic indicator. *J Pathol* 2001;**195**:435-42.

AIMS: Oesophageal basaloid squamous cell carcinoma (BSCC) is uncommon and has been reported to have a worse prognosis than squamous cell carcinomas (SCCs), but this tumour has not been fully characterized. The aim of the present study was to analyse the clinicopathological features of a large cohort of patients with oesophageal BSCC treated at a single institution.

METHOD: The pathology of 756 primary oesophageal cancers treated between January 1989 and December 1998 was reviewed. Tumours that fulfilled the diagnostic criteria of BSCC were identified and were compared with SCC. Their expression of MIB-1, DNA ploidy, and telomerase activity were also studied.

RESULTS: Thirty Chinese patients (25 men and five women) with BSCC were found, comprising 4% of patients with oesophageal cancer treated by surgical resection in the study period. Their median age was 67 years (range 40-78 years). Dysphagia was usually the main presenting symptom. Other concomitant malignant tumours were seen in three patients and para-neoplastic glomerulopathy in one. Five tumours were located in the upper third, 19 in the middle third, and six in the lower third. The median length was 5.8 cm (range 2-12 cm). The median MIB-1 score of BSCC was 750 (range 400-858) and was higher than that of SCC ($p=0.003$). The primary tumour and metastatic BSCC were aneuploid, as detected by flow cytometric analysis in nine patients. Telomerase activity was positive in 95% (19 out of 20) of the cases analysed. The 5-year survival of patients with BSCC was 12%. Distant metastases were seen in 53% ($n=16$); lung and liver were

the most common sites. The median survival of patients with tumours which had a high level of telomerase activity was significantly shorter than those with low levels of telomerase activity (1 vs. 27 months) ($p=0.001$). The median survival of patients with BSCC and SCC was 26 and 16 months, respectively ($p=0.3$).

CONCLUSION: In conclusion, BSCC has distinctive clinicopathological features and its long-term prognosis is no worse than SCC. The level of telomerase activity may have a prognostic role.

6. Lam KY, Law SYK, Chan KW, Yuen MC. Glomerulonephritis associated with basaloid squamous cell carcinoma of the oesophagus. *Scand J Urol Nephrol* 1998;**32**:61-63.

AIMS: To report the presence of glomerulonephritis in basaloid squamous cell carcinoma of the oesophagus.

METHOD: A 70-year-old man presented with rash, microscopic haematuria and proteinuria. He was subsequently found to have basaloid squamous carcinoma of the oesophagus. The renal biopsy was studied.

RESULTS: The renal biopsy showed mesangial proliferative glomerulonephritis with mesangial IgA deposition. This possible association between IgA nephropathy and oesophageal basaloid squamous carcinoma has never been reported.

CONCLUSION: Basaloid squamous cell carcinoma of the oesophagus could be associated with glomerulonephritis.

7. Lam KY, Law SYK, Loke SL, Fok M, Ma LT. Double sarcomatoid carcinomas of the oesophagus. *Path Res Pract* 1996;**192**:604-9.

AIMS: Sarcomatoid carcinoma of the oesophagus is uncommon. A case of double sarcomatoid of the oesophagus is reported.

METHOD: A case of double sarcomatoid carcinomas was identified in the oesophagus of a 48-year-old man. The features of multiple primary tumours and sarcomatoid carcinoma of the oesophagus are also reviewed.

RESULTS: This is the fourth case of multiple primary sarcomatoid carcinomas of the oesophagus and the first case with detailed pathological features presented.

CONCLUSION: Sarcomatoid carcinomas can occur in the oesophagus and multiple tumours can occur.

8. Lam KY, Law S, Tung PH, Wong J. Oesophageal small cell carcinoma:

clinicopathologic parameters, p53 overexpression, proliferative marker, and their impact on pathogenesis. *Arch Pathol Lab Med* 2000;**124**:228-233

AIMS: To evaluate the clinicopathological features and the roles of p53 and MIB-1 in oesophageal small cell carcinoma.

METHOD: Twenty patients (14 men and 6 women) with oesophageal small cell carcinoma treated in our hospital from 1982 through 1996 were studied. The clinicopathological features, treatment received, and survival data of these patients were documented. Representative tissue was collected from each tumour, and immunohistochemical preparations for p53 protein and MIB-1 were made.

RESULTS: Small cell carcinoma accounted for 1.3% of all oesophageal malignant tumours. The median age of patients at presentation was 60 years. On gross examination, the tumours were large ulcerative lesions (median length, 7.5 cm). In 17 patients in whom p53 immunohistochemical study was performed, p53 protein was detected in 65% (9 of 17). All stage IV tumours were negative for p53 expression. The median tumour cell MIB-1 score was high at 855 (range, 810-964) positive cells per 1000. Overall median survival was 3.4 months. In patients who underwent chemotherapy, there was significant response.

CONCLUSION: Oesophageal small cell carcinoma is an aggressive tumour. Over-expression of p53 is associated with early stages of carcinogenesis. The high proliferative index, as defined by the MIB-1 immunohistochemical method, may be related to aggressive behaviour and high sensitivity to chemotherapy and radiotherapy.

9. Law S, Lam KY, Chu KM, Wong J. Specialized intestinal metaplasia and carditis at the gastroesophageal junction in Chinese patients. *Am J Gastroenterol*; 2002;**97**:1924-29.

AIMS: Specialized intestinal metaplasia (SIM) is often found at a normal-looking gastroesophageal junction on routine biopsy. The prevalence of SIM in Asian populations has not been recorded. Its significance is also unclear. The objective of the study was to document the prevalence of SIM at the gastroesophageal junction in a Chinese population undergoing endoscopy.

METHOD: Biopsies were taken at the gastroesophageal junction in 145 patients, both at the squamocolumnar junction and immediately below in the gastric cardia. Specimens were examined for the type of epithelium (squamous, cardiac, and fundic), the presence of SIM, and *Helicobacter pylori* (*H. pylori*).

RESULTS: Of 145 patients who underwent endoscopy, 136 had a normal-looking gastroesophageal junction. Cardiac epithelium was found in 100 patients. Of these 100 patients, SIM was documented in 34% of patients and carditis in 20%. Patients with SIM were older compared with those without SIM (mean age 62 yr and 56 yr, $p = 0.035$). Carditis was more prevalent in patients with SIM. It was present in 11 out of 34 patients who had SIM (32.4%) compared with nine in 66 patients (13.6%) without SIM, $p = 0.036$. When carditis was found, *H. pylori* was present at the cardia in 40% of patients (eight of 20) compared with only 18% (14 of 80) in those without carditis, $p = 0.039$.

CONCLUSION: SIM is prevalent at the gastroesophageal junction in Chinese patients undergoing endoscopy and is associated with carditis. Carditis in turn may be related to *H. pylori* infection.

10. Lam KY, Law S, Wong J. malignant melanoma of the oesophagus: clinicopathological features, lack of p53 expression and steroid receptors and a review of the literature. *Eur J Surg Oncol* 1999;**25**:168-72.

AIMS: Fortunately, primary malignant melanoma of the oesophagus is a rare entity. The aims of this study were to evaluate the clinicopathological features, p53 over-expression and steroid receptors in oesophageal melanomas.

METHOD: Melanomas reported during a 15-year period (1982-1996) in the Queen Mary Hospital were studied. The clinicopathological features and survival data of patients with oesophageal melanomas were noted. Representative tissue was collected from each tumour and immunohistochemical preparations for HMB-45, p53, oestrogen and progesterone receptors were made.

RESULTS: Three cases of primary malignant melanoma of the oesophagus were found. They accounted for 3% of melanomas and 0.2% of oesophageal cancers diagnosed. The melanomas were fusiform and large at the time of resection. All three patients died of their malignancy within 9 months of operation. The tumours stained positive for HMB-45 and were negative for p53, oestrogen and progesterone receptors. From previous reports, 154 oesophageal melanomas were documented. The tumours were fusiform, large, often pigmented and located in either the middle or lower oesophagus. Although many oesophageal melanomas presented at early stages (stages I or II), their biological behaviour was aggressive. The 5-year survival rate was 5.7%.

CONCLUSION: Melanoma of the oesophagus is an aggressive tumour. There is no evidence for the p53 gene and female sex hormones having a role in the development or progression of the tumour.

11. Lam KY. Oesophageal mesenchymal tumours: clinicopathological features and absence of Epstein-Barr virus. *J Clin Pathol* 1999;**52**:758-60.

AIMS: Studies have suggested that the Epstein-Barr virus (EBV) is associated with smooth muscle tumours in patients with human immunodeficiency virus and in organ transplant recipients. The aims of the study are to report our experience on oesophageal mesenchymal tumours and to determine whether EBV is associated with these tumours.

METHOD: 40 sporadic oesophageal mesenchymal tumours were studied and their diagnosis confirmed on pathological review and immunohistochemical studies. Formalin fixed, paraffin was embedded tissues from these tumours were analysed for EBV using in situ hybridisation for two messenger RNA (mRNA) probes, EBER and BamH1 W.

RESULTS: The oesophageal mesenchymal tumours comprised 36 leiomyomas, two undifferentiated stromal tumours, and two gastrointestinal autonomic nerve tumours (GANTs). Median age of the patients with leiomyoma (26 men, 10 women) was 62 years (range 30 to 85) and 81% of them had an asymptomatic lesion. The median longitudinal size was 1.2 cm. Multiple leiomyomas were seen in 11% of the patients and calcification was noted in one tumour. Coexisting squamous cell carcinoma was found in one third of cases. The stromal tumours were small, asymptomatic, and located in the lower third of the oesophagus, while the GANTs were large, symptomatic, and found in the upper third of the oesophagus. EBV mRNAs were not detected in all these tumours.

CONCLUSION: The clinicopathological features of oesophageal leiomyoma, undifferentiated stromal tumour, and GANT were different. Some oesophageal leiomyomas were associated with oesophageal squamous cell carcinomas. EBV is not associated with sporadic oesophageal mesenchymal tumours.

12. Lam KY, Law SYK, Chu KM, Ma LT. Gastrointestinal autonomic nerve tumour of the esophagus: a clinicopathologic, immunohistochemical, ultrastructural study of a case and review of the literature. *Cancer* 1996;**78**:1651-59.

AIMS: Gastrointestinal autonomic nerve tumour (GANT) is a rare tumour that is supposed to originate from the enteric autonomic plexus. The tumour is a subgroup of the gastrointestinal stromal tumour that usually occurs in the stomach and small intestine.

The aim is to report a case in the oesophagus.

METHOD: An intramural tumour located in the upper third of the oesophagus of a 62-year-old Chinese female is reported. The tumour was removed by a three-phased esophagogastrectomy because of its large size. The tumour measured 6.5 cm x 5 cm x 4 cm. Its tissues were sampled, examined by light microscopy, immunohistochemistry, and electron microscopy.

RESULTS: The tumour was vaguely encapsulated but had foci of partial infiltration of the capsule. It was comprised of spindle cells with moderate nuclear pleomorphism. The mitotic count was less than 1 per 10 high-power field. The tumour stained positive for vimentin, neuron specific enolase (NSE), and S-100 protein, and was negative for cytokeratins, synaptophysin, chromogranin, neurofilaments, muscle markers, HMB45, and CD34. Ultrastructural study revealed that the tumour had cytoplasmic processes interdigitated in a complex fashion that were held together by primitive junctions but not invested in basal lamina. Many neurosecretory granules and neurotubules were also noted. The diagnosis was GANT of the oesophagus. From previous reports, 43 cases (25 males, 18 females) of GAN tumour of other locations have been documented. The tumours were located almost exclusively in the stomach and small intestine; rare cases

arose primarily in the retroperitoneum and mesentery. Some of these GANTs were observed in patients with Carney's triad (three cases), neurofibromatosis (two cases), and adrenal ganglioneuroma (one case). It appears that the biological behaviour of GANTs is aggressive but there are too few reports on which to conclude anything about their prognosis. The tumours are usually large, with low mitotic rate, and are positive for NSE and negative for muscle markers.

CONCLUSION: To the authors' knowledge, this is the first time that GAN is described in the oesophagus. The diagnosis can be made only on the basis of characteristic ultrastructural features.

13. Lam KY, Ma LT, Wong J. Measurement of extent of spread of oesophageal squamous carcinoma by serial sectioning. *J Clin Pathol* 1996;**49**:124-9.

AIMS: (1) To examine the prevalence and extent of intramural metastasis in squamous cell carcinomas of the oesophagus so as to delineate the resection margins for these tumours; (2) to devise an appropriate method for measurement of these lesions which takes into account of the contraction of the specimens after resection.

METHOD: Oesophagectomy specimens were prospectively collected from 96 patients (87 males, nine females) with primary oesophageal squamous cell carcinoma. The sizes of the tumours were measured in situ, after resection and after application of muscle relaxant (to regain their in situ length). The specimens were then serially sectioned for histological examination.

RESULTS: The sizes of the tumours measured after application of muscle relaxant roughly corresponded to those measured in situ. Intramural metastasis was observed in 26% of the cases. Sixty four per cent (16 cases) of these were on the oral side, 72% (18 cases) on the gastric side, and 25% (nine cases) on both sides of the tumours. The most distant extent of intramural metastasis from the primary tumour was from 0.5 cm to 7.7 cm (mean = 3.4 cm) on the oral side, and 0.5 to 9.5 cm (mean 4 cm) on the gastric aspect of the tumour. Intramural metastasis was seen only in patients in whom the primary cancer had deep muscle infiltration. Multiple neoplastic lesions could be detected in 33% of the patients. Both intramural metastasis and multiple neoplastic lesions were associated with extensive lymph node infiltration.

CONCLUSION: Intramural metastasis was frequently observed in oesophageal squamous cell carcinoma. This implies that excision with wide margins should be considered for local control of the disease.

14. Lam KY, Law S, Ma LT, Ong SK, Wong J. Pre-operative chemotherapy for squamous cell carcinoma of the oesophagus: do histological assessment and p53 over-expression predict chemo-responsiveness? *Eur J Cancer* 1997;**33**:1221-25.

AIMS: Pre-operative chemotherapy is increasingly used in the treatment of oesophageal carcinoma. However, no features have been identified which can reliably predict a positive response to chemotherapy. The aim of this study was to examine whether histological features and p53 over-expression could predict such response.

METHOD: Pre-chemotherapy endoscopic biopsies from 55 patients, who subsequently completed two courses of chemotherapy followed by surgical resection, were studied. Patients were classified into responders and non-responders according to clinical and pathological findings. Pathological features of the endoscopic biopsies examined included adequacy of the tumour tissue, histological grade, degree of keratinisation, histologic patterns, mitotic rates and nuclear pleomorphism. Biopsy specimens were also tested for p53 over-expression using p53 protein specific mouse monoclonal antibody DO-7 on paraffin sections. Histologic features and p53 expression were correlated to chemo-responsiveness.

RESULTS: 76% (42 of 55) of patients had sufficient biopsy tissue for assessment. Response to chemotherapy was evident in 64% (n = 27) of patients. None of the non-responders had tumours with high-grade nuclear pleomorphism compared with 37% (10 of 27) of responders (P = 0.01). All patients with high-grade nuclear pleomorphism responded to chemotherapy. No significant differences were found between the responders and non-responders with respect to tumour differentiation (P = 0.7), degree of keratinisation (P = 0.3) and mitotic rates (P = 0.8). Overall, p53 over-expression was

noted in 67% (28 of 42) of patients. This was more prevalent in non-responders (12/15) compared to responders (16/27), but this was not statistically significant ($P = 0.08$). The degree of p53 over-expression had no significant relationship with responsiveness to chemotherapy. High-grade nuclear pleomorphism, identified on pre-treatment biopsy specimens, correlated with response to chemotherapy, whereas p53 over-expression did not correlate with response. Improved tissue sampling and further investigations should be done so that the assessment of pre-chemotherapeutic endoscopic biopsies can have significant impact on clinical decision.

CONCLUSION: High-grade nuclear pleomorphism in oesophageal carcinomas was correlated to chemo-responsiveness of the tumour.

15. Hu YC, **Lam KY**, Wan TSK, Fang WG, Ma ESK, Chan LC, Srivastava G.

Establishment and characterization of HKESC-1, a new cancer cell line from human oesophageal squamous cell carcinoma. *Cancer Genet Cytogenet* 2000;**118**:112-20.

AIMS: The establishment of an oesophageal cancer cell line can facilitate the search for molecular mechanisms involved in oesophageal carcinogenesis. The aim is to establish a cell line from oesophageal squamous cell carcinoma.

METHOD: A new human cancer cell line, HKESC-1, was established from a primary moderately-differentiated squamous cell carcinoma of the oesophagus from a 47-year-old Hong Kong Chinese man. The pathological characteristics (morphology, immunohistochemical, and electron microscopic studies), the tumourigenecity in nude mice, the cytogenetic features, the DNA ploidy, and telomerase activity of the cell line were investigated.

RESULTS: The HKESC-1 cells have been maintained continuously in vitro for more than 16 months and passaged over 96 times. HKESC-1 cells grow as a monolayer, with a doubling time of 46 hours. The HKESC-1 cells are of a squamous epithelial origin, as shown by their immunopositivity with the anti-cytokeratin antibodies and ultrastructural demonstration of tonofilaments and desmosomes. The HKESC-1 cells possess characteristics of malignancy because they are highly tumorigenic in nude mice and have strong telomerase activity. The HKESC-1 cells had an aneuploid DNA content, as demonstrated by flow cytometric analysis. Cytogenetic analysis revealed hyperdiploidy of greater than 50 in 80% of analyzable metaphases. Chromosome gains and losses were common, and loss of the Y chromosome was a consistent numerical aberration.

Additionally, many structural chromosomal abnormalities were encountered, with frequent breakpoints at 1p32, 7p22, 7q34, and 20q13.

CONCLUSION: This newly established cell line serves as a useful model for studying the molecular pathogenesis, and testing new therapeutic reagents for oesophageal squamous cell carcinoma.

16. Tang JCO, Wan TSK, Wong N, Pang E, **Lam KY**, Law SYK, Chow LMC, Ma ESK, Chan LC, Wong J, Srivastava G. Establishment and characterization of a new xenograft-derived human oesophageal squamous cell line SLMT-1 of Chinese origin. *Cancer Genet Cytogenet* 2001;**124**:36-41.

AIMS: To establish and characterise an oesophageal tumour cell line from Hong Kong Chinese.

METHOD: A new human oesophageal cancer cell line, named SLMT-1, was established from a nude-mouse xenograft of a well-differentiated oesophageal squamous cell carcinoma (ESCC) of the lower oesophagus from a male Hong Kong Chinese patient. SLMT-1, passaged over 34 times and with a doubling time of 31 hours, has the microscopic features of epithelial cells with adherent growth as a monolayer.

RESULTS: The general biologic properties of SLMT-1 cells were characterized by (1) a positive test of tumorigenicity obtained by injecting cells subcutaneously into athymic nude mice and observing their development into well-differentiated squamous cell carcinoma; (2) immunohistochemical staining using antibodies (AE1/AE3, CAM5.2 and MAK 6) which show the presence of cytokeratin intermediate filaments; and (3) electron microscopy demonstrating the morphologic features of epithelial cells with the presence of desmosomes. The cytogenetic abnormalities found in both the primary culture and SLMT-1 included der(1;14)(q10;q10), add(1)(p1?), +1, +2, del(3)(q11), +6, +7, i(8)(q10), +8, +10, +11, -13, -15, +16, +17, -18, -19, -Y and marker chromosomes. Additional changes observed in the 34th passage included gains as well as losses of both numerical and structural abnormalities. Comparative genomic hybridization (CGH) indicated copy

number gains on chromosomal regions 3q32-qter, 5p, 8p12-p11.2, 11q13-q22 and 13q22-qter, and loss of the Y. The gains of 8p12-p11.2 in SLMT-1 cells are novel to ESCC.

CONCLUSION: Based on its distinct and common characteristics, the SLMT-1 cell line serves as a useful tool for studying the molecular and genetic basis of the pathogenesis of ESCC.

17. Hu YC, **Lam KY**, Law SYK, Wan TSK, Ma ESK, Kwong YL, Chan LC, Wong J, Srivastava G. Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human oesophageal squamous cell lines. *Cancer Genet Cytogenet* 2002;**135**:120-27.

AIMS: The establishment of oesophageal cancer cell lines can facilitate the search for molecular mechanisms underlying its pathogenesis. The objective is to establish two oesophageal cancer cell lines.

METHOD: Two novel human oesophageal squamous cell carcinoma (ESCC) cell lines, HKESC-2 and HKESC-3, were established from a moderately differentiated ESCC of a 46-year-old Chinese woman and a well-differentiated ESCC of a 74-year-old Chinese man, both from Hong Kong. The pathological characteristics (morphological, immunohistochemical, and electron microscopic studies), tumorigenicity in nude mice, cytogenetic features, and DNA ploidy of the two cell lines were investigated.

RESULTS: The two cell lines have been maintained in vitro for more than 17 months and passaged over 85 times for HKESC-2 and 58 times for HKESC-3. Both grew as monolayers, with a doubling time of 24 hours for HKESC-2 and 48 h for HKESC-3. Their squamous epithelial nature was authenticated by their strong immunopositivity with the anti-cytokeratin antibodies and the ultrastructural demonstration of tonofilaments and desmosomes. They are tumorigenic in nude mice and had DNA aneuploidy. G-banding cytogenetic analysis showed hyperdiploidy in HKESC-2 and near-tetraploidy in HKESC-3. Frequent breakpoints were noted at 1p22, 1p32, and 9q34 in HKESC-2 and at 1p31, 3p25, 3p14, 6q16, 6q21, 8p21, 9q34, 13q32, and 17q25 in HKESC-3. Comparative genomic hybridization analysis found that chromosomal gains were at 3q24-qter, 5q21-

qter, 8q11-qter, 13q21-q31, 17q11-qter, 19, 22q22 for HKESC-2 and at 3q13-qter, 5p, 6p, 9q21-qter, 10q21-q22, 12q15-pter, 14q24-qter, 16, 17q24-qter, 20 for HKESC-3.

Chromosomal losses were at 3p13-pter, 18q12-qter for HKESC-3.

CONCLUSION: These two newly established cell lines will be useful tools in the study of the molecular pathogenesis and biological behaviour of ESCC cells and for testing new therapeutic reagents for ESCC in the future.

18. Lam KY, Law SYK, So MKP, Fok M, Ma LT, Wong J. Prognostic implication of proliferative markers MIB-1 and PC 10 in oesophageal squamous cell carcinoma. *Cancer* 1996;**77**:7-13.

AIMS: Proliferative markers are related to tumour behaviour. The commonly used markers are proliferating cell nuclear antigen (PCNA) and Ki-67. The aim of this study is to evaluate the usefulness of MIB-1 (for Ki-67) and PC10 (for PCNA) in the assessment of the clinicopathological features and prognosis in patients with oesophageal squamous cell carcinoma.

METHOD: One hundred patients (88 males, 12 females; mean age, 63 years [range, 39 to 83 years]) with surgically resected oesophageal squamous cell carcinoma (32 well differentiated, 51 moderately differentiated, and 17 poorly differentiated) were studied. The clinicopathological features and survival data of these patients were noted. Representative tissue was collected from each tumour and immunohistochemical preparations for MIB-1 and PC10 were made.

RESULTS: The percentages of cells that tested positive for PC10 and MIB-1 were much higher in tumour cells than in non-neoplastic cells. The pattern of expression of both markers varied with the differentiation of the tumour. The results observed with MIB-1 staining were better than those with PC10; because MIB-1 had less background staining, as well as stronger and more uniform positive signals compared with PC10. Thus, further investigation was performed on MIB-1-stained sections. The tumour cell MIB-1 scores ranged from 169 to 964 positive cells per 1000 cells (mean 598 +/- 211; median, 636). Although it was significantly associated with the differentiation of the tumour ($P = 0.0001$), the score had no significant relationship to the tumour size, location, or stage, or

to the patients' age and sex. The prognosis depended on the size and stage of the lesion. In Stage III lesions (n = 83), patients with MIB-1 scores below 300 had longer actual survival rates than those with a score of 300 or above. However, the survival rates of patients in the latter group were better if the greatest dimension of the tumour diameter was 7.5 cm or less.

CONCLUSION: Proliferative activity in oesophageal squamous cell carcinoma, as defined by the MIB-1 immunohistochemical method, is significantly related to tumour differentiation. It is also potentially valuable as a prognostic marker in addition to its use in tumour staging and size.

19. Law AKW, Lam KY, Lam FK, Wong TKW, Poon JLS, Chan FHY. Image analysis system for assessment of immunohistochemistry stained proliferative marker (MIB-1) in oesophageal squamous cell carcinoma. *Comp Methods Programs Biomed* 2003;**70**:37-45.

AIMS: The prognosis of oesophageal cancer patients is related to the portion of MIB-1 positively stained tumour nuclei. In this study, an image analysis system was developed based on LEICA Image Processing and Analysis System to reduce the subjective, tedious and inaccurate manual counting of nuclei staining.

METHOD: Representative oesophageal cancer tissues were collected and immunohistochemical preparations of MIB-1 were made. The MIB-1 positive nuclei in these tumours were assessed by quantitative counting, semi-quantitative counting, and three computer assessment methods using LEICA QWIN PRO.

RESULTS: Our results showed that computer assessment methods were reliable and consistent. The procedure using the system could be accomplished within 15 min. Overlapped or missed counting of nuclei by the observer was eliminated.

CONCLUSION: The image analysis system can really assist experts in obtaining reliable data for the prognosis of oesophageal cancer patients quickly.

20. Lam KY, Ma L, Law SYK, Cheung LKN, Luk TF, Wong J. Use of flow cytometry in the analysis of stage III squamous cell carcinoma of the oesophagus and its association with MIB-1. *J Clin Pathol* 1996;**49**:975-78.

AIMS: To examine the prognostic and pathobiological importance of DNA content in oesophageal squamous cell carcinomas in Hong Kong Chinese subjects; to evaluate its association with the immunohistochemical proliferative marker MIB-1.

METHOD: Paraffin wax embedded tumour tissue and adjacent normal tissue (control tissue) samples from 45 resected stage III oesophageal squamous cell carcinomas were studied using flow cytometric analysis. The DNA content and the clinicopathological data of these patients were analysed together with the MIB-1 labelling index.

RESULTS: DNA aneuploidy was present in 14 (31%) of the 45 cases. However, the DNA content did not correlate significantly with the age, sex, or survival of the patients, nor the length, location, differentiation and MIB-1 labelling index of the oesophageal carcinomas. The synthetic (S) phase fraction of diploid tumours bore no relation to the patients' survival or MIB-1 score.

CONCLUSION: Flow cytometry was not as useful as the MIB-1 labelling index in predicting the biological characteristics of the tumours and the prognosis of patients with oesophageal squamous cell carcinomas. This study does not support the routine use of DNA flow cytometric analysis in oesophageal cancers.

21. Lam KY, He D, Ma L, Zhang D, Ngan HYS, Wan TSK, Tsao SW. Presence of human papillomavirus in oesophageal squamous cell carcinomas of Hong Kong Chinese and its relationship with p53 gene mutation. *Hum Pathol* 1997;**28**:657-63.

AIMS: There is no scientific study that has investigated the association between human papilloma virus (HPV) and p53 mutation in Hong Kong Chinese patients with oesophageal cancers. The aim of this survey is to evaluate the prevalence and relationship of HPV and p53 mutation in these patients with oesophageal squamous cell carcinomas.

METHOD: Fresh tissues from the resected specimens of 70 Chinese patients (59 men, 11 women) with primary oesophageal squamous cell carcinomas (20 well-differentiated, 36 moderately differentiated, and 14 poorly differentiated squamous cell carcinomas) were tested for the presence of HPV and p53 mutation using the polymerase chain reaction (PCR), single-strand conformational polymorphism (SSCP) analysis, and DNA sequencing.

RESULTS: No HPV type 18 was detected, whereas HPV type 16 was identified in 8.6% (6 of 75) of the cases. p53 mutation was found in 44% (31 of 70) of the tumours. The mean ages of HPV-positive and HPV-negative groups of patients were 55 and 64 years, respectively ($P = .046$, t-test). There was no correlation between the prevalence of HPV and p53 mutation in these tumours. The presence of HPV and p53 also had no relation to the sex of the patients or to the grade of the carcinomas. It is

CONCLUSION: The overall low prevalence of HPV in oesophageal carcinomas may suggest that the virus may not play an important role in the pathogenesis of these tumours in Hong Kong Chinese patients. Also, p53 mutation and integrated HPV DNA are not mutually exclusive in oesophageal cancer.

22. Lam KY, Srivastava G, Leung ML, Ma L. Absence of Epstein-Barr virus in oesophageal squamous cell carcinoma: a study of 74 cases using in-situ hybridization. *J Clin Pathol Mol Pathol* 1995;**48**:M188-90.

AIMS: The association between Epstein-Barr virus (EBV) and oesophageal cancers has seldom been investigated. The aim of this study is to identify the possible role of EBV in oesophageal squamous cell carcinomas of Chinese patients.

METHOD: Formalin fixed and paraffin embedded tissues of 74 cases of oesophageal squamous cell carcinomas (28 well-differentiated, 27 moderately-differentiated and 18 poorly-differentiated squamous cell carcinomas) were analyzed for EBV using in situ hybridisation for EBV-encoded small RNAs (EBERs).

RESULTS: EBV was only demonstrated in a few lymphocytes adjacent to the tumour epithelia in 19% (14 cases) of oesophageal carcinomas, but the adjacent non-pathological oesophageal tissue were negative for EBV.

CONCLUSION: The results suggest that EBV does not play a major role in the aetiology of oesophageal squamous cell carcinoma.

23. Lam KY, Loke SL, Shen XC, Ma LT. Cytokeratin expression in non-neoplastic oesophageal epithelium and squamous cell carcinoma of the oesophagus. *Virchows Arch A* 1995;**426**:345-49.

AIMS: The study was undertaken to assess the pattern of expression of these keratins in oesophageal tumours and its relation to the degree of differentiation.

METHOD: The expression of cytokeratins (CK) 19, 8, 18, 13, 10 and 7 was examined in 35 cases of squamous cell carcinomas of the oesophagus (10 well-differentiated, 13 moderately-differentiated, and 12 poorly-differentiated) and the adjacent mucosa by means of a panel of monoclonal antibodies on frozen sections.

RESULTS: The normal oesophageal epithelia expressed CK19 in 86%, CK18 in 17% and CK13 in 14% of cases. CK8, CK10 and CK7 immunoreactivity was not observed. The tumours expressed CK19 in 86%, CK8 in 46%, CK18 in 97%, CK13 in 83%, CK10 in 34% and CK7 in 29% of cases. Thus, the so-called simple epithelial markers CK18 and CK19 occurred in the majority of oesophageal squamous cell carcinomas. CK13 (the so-called non-keratinizing squamous epithelial marker) was only infrequently demonstrated in the non-neoplastic oesophageal mucosa, and its expression was more frequent in carcinomas. CK10 was not demonstrated in non-neoplastic mucosa, but was mostly associated with well-differentiated carcinomas.

CONCLUSION: We conclude that the pattern of expression of cytokeratins in oesophageal carcinomas is different from that in normal oesophageal epithelia and varies with differentiation.

24. Lam KY, Tsao SW, Zhang D, Law S, He D, Ma L, Wong J. Prevalence and predictive value of p53 mutation in patients with oesophageal squamous cell carcinomas: a prospective clinicopathological study and survival analysis of 70 patients. *Int J Cancer* 1997;**74**:212-19.

AIMS: To study the prevalence and predictive value of p53 mutation in patients with oesophageal squamous cell carcinoma.

METHOD: The tissues from 70 Chinese patients with oesophageal squamous cell carcinoma were prospectively collected to study for the pattern of p53 mutations and its relationship with clinico-pathological features and prognosis using immunohistochemistry, polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis and DNA sequencing.

RESULTS: p53 over-expression and p53 mutations were detected in 73% and 44% of the patients. These p53 aberrations had no relationship with the patient age, sex, smoking/drinking habits and tumour site, size or stage. The p53 over-expression was more intense in moderately/poorly-differentiated squamous cell carcinomas. Thirty-three p53 mutations were noted in 31 patients; 18.2% in exon 5, 15.2% in exon 6, 33.3% in exon 7 and 33.3% in exon 8. Mutations were primarily point mutations and common in codons 248, 273 and 285. There were 46% transversions, 36% transitions and 18% frameshift. The survival of the patients depended mainly on the extent of resection. In patients with stage III oesophageal cancer, the median survival of those with p53 Mutations was 6.8 months whereas those without was 12.5 months. The results were of clinical importance although the value did not reach statistical significance.

CONCLUSION: There was a definite role of p53 mutations in the pathogenesis of oesophageal squamous cell carcinomas. p53 mutations were not synonymous with p53

over-expression. The distribution of p53 mutations in oesophageal cancers suggested that the etiologic contribution might be complex and probably involve different exogenous and endogenous exposures. p53 mutations also appear to play a role in predicting the survival of patients with stage III oesophageal squamous cell carcinomas.

25. Lam KY, Law S, Lo T, Tung HM, Wong J. The clinicopathological significance of p21 and p53 expression in oesophageal squamous cell carcinoma: an analysis of 153 patients.

Am J Gastroenterol; 1999;**94**:2060-68.

AIMS: The aim of this study was to examine the clinicopathological role of p21 and p53 in oesophageal squamous cell carcinomas.

METHOD: The expression of p21 and p53 proteins in 153 Chinese patients (131 men, 22 women) with resected oesophageal squamous cell carcinomas was investigated by the immunohistochemical method. Correlation between p21 and p53 expression and clinicopathological features was examined.

RESULTS: The expression of p21 and p53 was detected in 70% and 64% of the tumours, respectively. The staining of p21 and p53 was also found in squamous carcinoma in situ, dysplasia, and non-tumour epithelium. p21 expression was often weak in the suprabasal cells and found in better differentiated tumours. There was no significant correlation between the expression of p21 and the abnormal accumulation of p53. The prognosis of the patients depended on the size, stage, and p21 expression of the lesion. In stage III lesions with tumour diameter ≤ 7.5 cm (n = 93), patients with loss of p21 expression had better survival. The survival rates of patients were worse if they had expression of both p21 and p53.

CONCLUSION: p21 and p53 had prognostic value for oesophageal squamous cell carcinomas. Loss of p21 expression was shown without p53 alternations, indicating that other mechanisms are also involved in turning off the gene. The pattern of p21 and p53 expression predicts an aggressive clinical course of oesophageal squamous cell carcinomas.

26. Lam KY, Tin L, Ma L. c-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. *Eur J Surg Oncol* 1998;**24**:431-35.

AIMS: c-erbB-2, an oncogene, is member of the growth factor receptor family. Its role in activation of oesophageal squamous cell carcinoma is poorly understood. The aim of this study was to evaluate the part played by c-erbB-2 in oesophageal squamous cell carcinoma in Hong Kong Chinese patients.

METHOD: We examined the expression of the c-erbB-2 oncoprotein in 104 oesophageal squamous cell carcinomas from 89 men and 15 women, ranging in age from 41 to 89 years (mean 63). C-ercB-2 expression was studied with monoclonal antibody, using an antigen retrieval method.

RESULTS: Focal c-erbB-2 membrane staining was present in 10 (10%) of 104 squamous cell carcinomas. Staining was also noted in the adjacent dysplastic epithelium (n=2) and non-tumour inflamed epithelium (n=2). In carcinomas, the c-erbB-2 membrane staining was identified only in superficial well-differentiated tumour cells and the expression did not predict biological behaviour.

CONCLUSION: We conclude that the c-erbB-2 oncoprotein is expressed in a portion of oesophageal squamous cell carcinomas and precursor lesions. This suggests that c-erbB-2 activation plays a certain role, mostly probably during the early stages, in carcinogenesis in oesophageal squamous cell carcinomas from Hong Kong Chinese patients.

27. Hu YC, Lam KY, Tang JCO, Srivastava G. Mutational analysis of the PTEN/MMAC1 gene in primary oesophageal squamous cell carcinomas. *J Clin Pathol Mol Pathol* 1999;**52**:353-56.

AIMS: To investigate whether PTEN/MMAC1 mutations play a role in the carcinogenesis of oesophageal squamous cell carcinoma.

METHOD: A panel of 33 primary oesophageal squamous cell carcinoma tumour samples and 20 corresponding morphologically normal tissues was examined for mutations in all nine exons of the PTEN/MMAC1 gene by means of polymerase chain reaction single strand conformational polymorphism analysis (PCR-SSCP) and direct DNA sequencing methods.

RESULTS: Only one of 33 oesophageal squamous cell carcinomas showed an aberrant SSCP band. Further sequencing analysis of this sample revealed an 802 -29 T-->C substitution in intron 7. PTEN/MMAC1 mutations were not found in the mutational "hot spot" in exon 5, even after direct sequencing of six oesophageal squamous cell carcinoma samples and three normal tissues. However, a deletion of one nucleotide T at position 492 +8 in intron 5 was seen in all samples.

CONCLUSION: These results suggest that PTEN/MMAC1 mutations do not play a major role in the carcinogenesis of oesophageal squamous cell carcinoma.

28. Chow V, Law S, **Lam KY**, Luk JM, Wong J. Telomerase activity in small cell oesophageal carcinoma. *Dis Esophagus* 2001;**14**:139-42.

AIMS: Small cell carcinoma of the oesophagus is a rare and aggressive malignant tumour. Telomerase activation is common in human cancers. There is a lack of data on telomerase activity in oesophageal small cell cancers. The present report studied the role of telomerase activity in oesophageal small cell carcinoma.

METHOD: The clinicopathological data of five patients with small cell carcinoma of the oesophagus who underwent primary surgical treatment between 1991 and 2000 were studied. Telomeric repeat amplification protocol assays were used to investigate telomerase activity in these tumours. The proliferative activity (MIB-1) and p53 expression of these tumours were also studied using immunohistochemistry and correlated with the telomerase activity.

RESULTS: All five small cell carcinomas showed detectable telomerase activity in the primary tumour. Two out of the five morphologically normal oesophageal mucosae adjacent to the primary tumour had detectable telomerase activity. There was no correlation between the p53 expression, tumour stage, survival of patients, and the presence of telomerase activity. High MIB-1 expression in oesophageal small cell carcinomas was associated with high telomerase activity.

CONCLUSION: Telomerase activation is common in small cell carcinoma of the oesophagus. This fact may find application in anti-telomerase treatment for this aggressive tumour.

29. Tang JCO, Lam KY, Law S, Wong J, Srivastava G. Detection of genetic alterations in oesophageal squamous cell carcinomas and adjacent normal epithelia by comparative DNA fingerprinting using inter-simple sequence repeat PCR. *Clin Cancer Res* 2001;**7**:1539-45.

AIMS: To detect the genetic alterations in oesophageal squamous cell carcinoma using a novel approach.

METHOD: In this study, we screened 19 oesophageal squamous cell carcinomas (ESCCs) for the detection of genetic alterations using inter-simple sequence repeat PCR, a DNA fingerprinting approach. Three simple repetitive unanchored primers representing tri- and tetranucleotide repeats [(GTG)(5), (GACA)(4), and (GATA)(4)] were used.

RESULTS: Evidence of gains and losses of chromosomal sequences were detected in all tumours (19 of 19 cases) for at least one of the primers. In 13 of these cases, apparently normal marginal epithelia adjacent to the tumours were also collected and examined. Eight of the 13 (62%) patients showed matching somatic mutations in the marginal epithelia adjacent to the tumours. Five of these 8 (63%) marginal epithelial samples were histologically normal, two were dysplastic, and one had extremely rare tumour cells. In 3 of these 13 (23%) cases, the profile bands were also seen to quantitatively increase in intensity, progressing from normal epithelia to marginal epithelia to tumours. Ten profile bands showing gains and one profile band showing loss in tumours compared with the corresponding normal epithelia were cloned, and their origins were determined by sequencing. The DNA sequence of one of the profile bands showing gain in the tumour could be matched to an expressed sequence tag sequence that has been mapped to the 7q22 region, a genomic amplification novel to ESCC. The sequence of the other profile

band showing gain in the tumour could be matched to a non-exonic sequence of chromosome 20, whereas the sequences of the remaining profile bands could not be matched with any known sequences after comparison with the genomic sequence data in the European Molecular Biology Laboratory and GenBank databases. The bona fide nature of the gains or losses of 11 profile bands in the original cases was confirmed by direct genomic PCR amplification. The frequencies of these specific gene alterations in tumours were then analyzed in a total of 60 ESCCs, which included 41 additional cases of ESCC. Significantly, 26 of 60 (43%) tumours showed the DNA amplification for the expressed sequence tag sequence of chromosome 7, whereas the frequency of other individual gene alterations ranged from 7% to 15%.

CONCLUSION: It is concluded that the inter-simple sequence repeat PCR strategy is adequate for the detection of somatic mutations in tumours, most of which are quantitative alterations in anonymous genomic sequences. This approach is also suitable for detection of somatic mutations preceding the onset of morphologically detectable neoplasia in ESCC.

30. Hu YC, Lam KY, Law S, Wong J, Srivastava G. Identification of differentially expressed in oesophageal squamous cell carcinoma (ESCC) by cDNA expression array: over-expression of Fra-1, Neogenin, Id-1 and CDC25B genes in ESCC. *Clin Cancer Res* 2001;**7**:2213-21.

AIMS: This study aims to identify differentially expressed genes in oesophageal squamous cell carcinoma (ESCC) through the use of a membrane-based cDNA array.

METHOD: Two newly established human ESCC cell lines (HKESC-1 and HKESC-2) and one corresponding to a morphologically normal, oesophageal epithelium tissue specimen, prospectively collected from the HKESC-2-related patient, were screened in parallel using a cDNA expression array containing gene-specific fragments for 588 human genes spotted onto nylon membranes.

RESULTS: The results of cDNA expression array showed that 53 genes were up-regulated 2-fold or higher and 8 genes were down-regulated 2-fold or higher in both ESCC cell lines at the mRNA level. Semiquantitative RT-PCR analysis of a subset of these differentially expressed genes gave results consistent with cDNA array findings. Four of the differentially expressed genes that belong to the categories of oncogenes/tumour suppressor genes (Fra-1 and Neogenin) and cell cycle-related genes (Id-1 and CDC25B) were studied more extensively for their protein expression by immunohistochemistry. The two ESCC cell lines and their corresponding primary tissues, 61 primary ESCC resected specimens and 16 matching, morphologically normal, oesophageal epithelium tissues were analyzed. The immunostaining results showed that Fra-1, Neogenin, Id-1, and CDC25B were overexpressed in both ESCC cell lines and their corresponding primary tumours at the protein level, validating the microarray

findings. The results of the clinical specimens showed that the Fra-1 gene was over-expressed in ESCC compared with normal oesophageal epithelium in 53 of 61 cases (87%), Neogenin in 57 of 61 cases (93%), Id-1 in 57 of 61 cases (93%), and CDC25B in 48 of 61 cases (79%). Furthermore, the expression of Fra-1, Neogenin, and Id-1 in ESCC correlated with tumour differentiation.

CONCLUSION: Overall, this study demonstrates that multiple genes are differentially expressed in ESCC and provides the first evidence that oncogenes Fra-1 and Neogenin and cell cycle-related genes Id-1 and CDC25B are over-expressed in ESCC.

31. Hu YC, Lam KY, Law S, Wong J, Srivastava G. Profiling of differentially expressed cancer-related genes in oesophageal squamous cell carcinoma (ESCC) using human cancer cDNA arrays: overexpression of oncogene MET correlates with tumour differentiation in ESCC. *Clin Cancer Res* 2001;**7**:3519-25.

AIMS: To examine the global gene expression of cancer-related genes in oesophageal squamous cell carcinoma (ESCC) through the use of Atlas Human Cancer Array membranes printed with 588 well-characterized human genes involved in cancer and tumour biology.

METHODS: Two human ESCC cell lines (HKESC-1 and HKESC-2) and one morphologically normal oesophageal epithelium tissue specimen from the patient of which the HKESC-2 was derived were screened in parallel using cDNA expression arrays. The array results were additionally validated using semi-quantitative PCR. The over-expression of oncogene MET was studied more extensively for its protein expression by immunohistochemistry in the two ESCC cell lines and their corresponding primary tissues and 61 primary ESCC resected specimens. Sixteen of these 61 ESCC cases also had available the corresponding morphologically normal oesophageal epithelium tissues and were also analyzed for MET expression. The clinicopathological features associated with over-expression of the MET gene were also correlated.

RESULTS: The results of cDNA arrays showed that 13 cancer-related genes were up-regulated ≥ 2 -fold (CDC25B, cyclin D1, PCNA, MET, Jagged 2, Integrin alpha3, Integrin alpha6, Integrin beta4, Caveolin-2, Caveolin-1, MMP13, MMP14, and BIGH3) and 5 genes were down-regulated ≥ 2 -fold (CK4, Bad, IGFBP2, CSPCP, and IL-1RA) in both ESCC cell lines at the mRNA level. Semi-quantitative RT-PCR analysis of

9 of these differentially expressed genes, including the MET gene, gave results consistent with cDNA array findings. The immunostaining results of the expression of MET gene showed that MET was over-expressed in both ESCC cell lines and their corresponding primary tumours at the protein level, validating the cDNA arrays findings. The results of the clinical specimens showed that the MET gene was over-expressed in ESCC compared with normal oesophageal epithelium in 56 of 61 cases (92%). Moreover, the over-expression of MET protein was more often seen in well/moderately differentiated than in poorly differentiated ESCC.

CONCLUSION: Multiple cancer-related genes are differentially expressed in ESCC, the oncogene MET is over-expressed in ESCC compared with normal oesophageal epithelium, and its protein over-expression correlates with tumour differentiation in ESCC.

32. Kwong D, Lam A, Guan X, Law S, Tai A, Wong J, Sham J. Chromosomal aberrations in oesophageal squamous cell carcinoma among Chinese: gain of 12p predicts poor prognosis after surgery. *Hum Pathol.* 2004;**35**:309-16

AIMS: To identify cytogenetic changes in oesophageal squamous cell carcinoma (ESCC) and to evaluate the importance of these changes by their correlation with clinical data.

METHOD: Sixty primary ESCC were evaluated for cytogenetic changes by comparative genomic hybridization. Recurrent chromosomal aberrations were correlated with stage and clinical outcome after esophagectomy.

RESULTS: Chromosomal aberrations were found in 52 (86.7%) cases. The most frequently detected chromosomal gains involved 3q (67.3%), 8q (57.7%), 5p (51.9%), 7q (28.8%), 15q (28.8%), 20p (21.1%), 20q (28.8%), 1q (26.9%), 7p (26.9%), 2p (23.1%) and 12p (23.1%). Chromosome 12p was most frequently involved in high level amplification. Six of the 12 cases with gain in 12p showed high level amplifications and the minimum overlapping region localized to 12pter-p13. The most frequently detected chromosomal loss involved 3p (46.2%), 4q (26.9%), 4p (23.1%), 3q (19.2%), 9p (17.3%), 19p (17.3%) and whole 13 (15.4%). No significant difference in early (I and II) and late (III and IV) stage distribution was found for recurrent chromosomal aberrations. Gain in 12p and loss in 3p were indicative of poor prognosis. Ten out of 12 (83.3%) patients with 12p gain and 19 out of 24 (79.2%) patients with 3p loss had relapse. Gain in 12p also predicted for significantly impaired relapse free survival.

CONCLUSION: Chromosomal aberrations are common in ESCC and gain in 12p is indicative of aggressive behaviour and poor prognosis.

D. Original and scholarly contribution to knowledge of publications

The publications are an original and scholarly contribution to knowledge because:

(a) the works are representative of the nature of the disease as they were performed in the largest referral centre in Hong Kong. A single team of specialist performed the clinical and laboratory tests. The centre is one the largest in the world operating on esophageal cancers

(b) the works were done on population that are unique (all are Chinese living in Hong Kong) and long-term follow-up data were available in nearly all the patients with esophageal cancers.

(c) many of the laboratory investigations were performed either on largest number of patients or being first reported in the esophageal cancers in the literature.

(d) the works were published in journals with high impact factor (13 publications have impact factor above 2 and two above 5). Many researchers in the field have quoted the published works (Up to December 2004, 11 publications have been quoted for more than 10 times and on publication been quoted for 40 times in the literature)

E. Themes in published works

The significance and coherence of the submitted material is considered under the following headings:

Epidemiology and clinicopathological features of oesophageal tumours

1. Prevalence and features of different types of oesophageal tumours
2. Clinical implication of histological assessment

Molecular biology of oesophageal tumours

1. Establishment of cell lines
2. Proliferative markers
3. Viral etiopathogenesis
4. Cytokeratin expression
5. Tumour-related genes
6. Gene mapping

I. Epidemiology and clinicopathological features of oesophageal tumours

1. Prevalence and features of different types of oesophageal tumours

Oesophageal tumour is very prevalent worldwide. The proportion of various histological subtypes of tumours is different in different regions. The clinicopathological features of these tumours were published in the following papers:

- **Lam KY, Law S, Wong J. Low prevalence of incidentally discovered and early-stage oesophageal cancers in a 30-year autopsy study. *Dis Esophagus* 2003;16:1-3.**
- **Lam KY, Ma L. Pathology of oesophageal cancers: local experience and current insights. *Chin Med J* 1997;110:459-64.**

These papers focused on the autopsy and biopsy findings of oesophageal cancers in a large cohort of patients. The prevalence of different histological types was noted. The commonest histological subtype in Hong Kong is squamous cell carcinoma. The tumours were usually at advanced stage at presentation.

- **Fegelman E, Law SYK, Fok M, Lam KY, Loke SL, Ma LT, Wong J. Squamous cell carcinoma of the esophagus with mucin-secreting component (mucoepidermoid carcinoma). *J Thoracic Cardiovasc Surg* 1994;107:62-67.**
- **Lam KY, Loke SL, Ma LT. Histochemistry of the mucin-secreting components in mucoepidermoid and adenosquamous carcinoma of the esophagus. *J Clin Pathol* 1993;46:1011-15.**

These two papers focused on the subtype of squamous cell carcinoma, mucoepidermoid or adenosquamous carcinoma. The prognosis was not significantly different from that of patients with squamous cell carcinoma or adenocarcinoma. The glandular component of this group of tumours histochemically differentiated in the direction of oesophageal glands. A mucin component should be identified for the diagnosis of the carcinomas.

- **Lam KY, Law S, Tung PH, Wong J. Oesophageal basaloid squamous cell carcinoma: an unique clinicopathological entity with telomerase activity as a prognostic indicator. *J Pathol* 2001;195:435-42.**
- **Lam KY, Law SYK, Chan KW, Yuen MC. Glomerulonephritis associated with basaloid squamous cell carcinoma of the esophagus. *Scand J Urol Nephrol* 1998;32:61-63.**

These two papers focused on oesophageal basaloid squamous cell carcinoma. The variant of squamous cell carcinoma had distinctive clinicopathological features. The level of telomerase activity may have a prognostic role in this tumour. Also, the disease can be associated with IgA nephropathy.

- **Lam KY, Law SYK, Loke SL, Fok M, Ma LT. Double sarcomatoid carcinomas of the esophagus. *Path Res Pract* 1996;192:604-9.**

Sarcomatoid carcinoma, a variant of squamous cell carcinoma, of the oesophagus is uncommon. Double sarcomatoid carcinomas were noted in the oesophagus.

- **Lam KY, Law S, Tung PH, Wong J. Esophageal small cell carcinoma: clinicopathologic parameters, p53 overexpression, proliferative marker, and their impact on pathogenesis. *Arch Pathol Lab Med* 2000;124:228-233.**

Small cell carcinoma of the oesophagus is a very aggressive tumour. Overexpression of p53 is common. The high proliferative index, as defined by the MIB-1 immunohistochemical method, may be related to aggressive behavior and high sensitivity to chemotherapy and radiotherapy.

- **Law S, Lam KY, Chu KM, Wong J. Specialized intestinal metaplasia and carditis at the gastroesophageal junction in Chinese patients. *Am J Gastroenterol*; 2002;97:1924-29.**

Adenocarcinoma of oesophagus is uncommon in Hong Kong. Intestinal metaplasia in the gastroesophageal junction is associated with adenocarcinoma. The paper documented that intestinal metaplasia was commonly found despite that adenocarcinoma is uncommon.

- **Lam KY, Law S, Wong J. malignant melanoma of the oesophagus: clinicopathological features, lack of p53 expression and steroid receptors and a review of the literature. *Eur J Surg Oncol* 1999;25:168-72.**

The paper found that primary malignant melanoma of the oesophagus was an uncommon, but very aggressive tumour.

- **Lam KY. Oesophageal mesenchymal tumours: clinicopathological features and absence of Epstein-Barr virus. *J Clin Pathol* 1999;52:758-60.**
- **Lam KY, Law SYK, Chu KM, Ma LT. Gastrointestinal autonomic nerve tumour of the esophagus: a clinicopathologic, immunohistochemical, ultrastructural study of a case and review of the literature. *Cancer* 1996;78:1651-59.**

These two papers focused on the mesenchymal tumours of the oesophagus. The oesophageal mesenchymal tumours were often leiomyomas. Less common tumours comprised undifferentiated stromal tumours and gastrointestinal autonomic nerve tumours. The clinicopathological features of them were different. Epstein Barr virus was not associated with sporadic oesophageal mesenchymal tumours.

2. Clinical implication of histological assessment.

Pathological assessments are important for the management of oesophageal cancers. The following papers described the importance of these assessments in the clinical settings:

- **Lam KY, Ma LT, Wong J. Measurement of extent of spread of esophageal squamous carcinoma by serial sectioning. *J Clin Pathol* 1996;49:124-9.**

This paper examined the extent of intramural metastasis and multiple tumours in cancer in the oesophagus by serial sectioning of the oesophagectomy specimens. Intramural metastasis was observed in 26% and multiple mucosal neoplastic lesions could be detected in 33% of the patients. Both intramural metastasis and multiple mucosal neoplastic lesions were associated with extensive lymph node infiltration. This implies that excision with wide margins should be considered for local control of the disease.

- **Lam KY, Law S, Ma LT, Ong SK, Wong J. Pre-operative chemotherapy for squamous cell carcinoma of the esophagus: do histological assessment and p53 over-expression predict chemo-responsiveness? *Eur J Cancer* 1997;33:1221-25.**

Pre-operative chemotherapy is increasingly used in the treatment of oesophageal carcinoma. High-grade nuclear pleomorphism, identified on pre-treatment biopsy specimens, correlated with response to chemotherapy, whereas p53 over-expression did not correlate with response. Thus, histological assessment has a role in predicting the response to chemotherapy in oesophageal carcinoma.

II. Molecular biology of oesophageal tumours

The molecular markers have potential roles to complement the traditional approaches in the management of oesophageal tumours. Unfortunately, there are only a few large-scale controlled trials to assess the roles of these molecular markers. The essence of the publications lies in the translation of research findings into clinical practice.

1. Establishment of cell lines

- **Hu YC, Lam KY, Wan TSK, Fang WG, Ma ESK, Chan LC, Srivastava G. Establishment and characterization of HKESC-1, a new cancer cell line from human oesophageal squamous cell carcinoma. *Cancer Genet Cytogenet* 2000;118:112-20.**
- **Tang JCO, Wan TSK, Wong N, Pang E, Lam KY, Law SYK, Chow LMC, Ma ESK, Chan LC, Wong J, Srivastava G. Establishment and characterization of a new xenograft-derived human oesophageal squamous cell line SLMT-1 of Chinese origin. *Cancer Genet Cytogenet* 2001;124:36-41.**
- **Hu YC, Lam KY, Law SYK, Wan TSK, Ma ESK, Kwong YL, Chan LC, Wong J, Srivastava G. Establishment, characterization, karyotyping, and comparative genomic hybridization analysis of HKESC-2 and HKESC-3: two newly established human oesophageal squamous cell lines. *Cancer Genet Cytogenet* 2002;135:120-27.**

These 3 papers described the works in establishment of 4 cell lines and the properties of cell lines, locally produced in Hong Kong. They were used for studies in the pathogenesis and properties of oesophageal cancer.

2. Proliferative markers

- **Lam KY, Law SYK, So MKP, Fok M, Ma LT, Wong J. Prognostic implication of proliferative markers MIB-1 and PC 10 in oesophageal squamous cell carcinoma. *Cancer* 1996;77:7-13.**
- **Law AKW, Lam KY, Lam FK, Wong TKW, Poon JLS, Chan FHY. Image analysis system for assessment of immunohistochemistry stained proliferative marker (MIB-1) in oesophageal squamous cell carcinoma. *Comp Methods Programs Biomed* 2003;70:37-45.**
- **Lam KY, Ma L, Law SYK, Cheung LKN, Luk TF, Wong J. Use of flow cytometry in the analysis of stage III squamous cell carcinoma of the esophagus and its association with MIB-1. *J Clin Pathol* 1996;49:975-78.**

These 3 papers described the application of proliferative markers in esophageal cancers.

Proliferative marker like MIB-1 was found to be useful in predicting the prognosis of oesophageal squamous cell carcinoma, and superior to PC-10 or flow cytometry. Image analysis can help in counting of MIB-1.

3. Viral etiopathogenesis

- **Lam KY, He D, Ma L, Zhang D, Ngan HYS, Wan TSK, Tsao SW. Presence of human papillomavirus in esophageal squamous cell carcinomas of Hong Kong Chinese and its relationship with p53 gene mutation. *Hum Pathol* 1997;28:657-63.**
- **Lam KY, Srivastava G, Leung ML, Ma L. Absence of Epstein-Barr virus in esophageal squamous cell carcinoma: a study of 74 cases using in-situ hybridization. *J Clin Pathol Mol Pathol* 1995;48:M188-90.**

In these papers, human papilloma virus was noted to be present in a small proportion of oesophageal squamous cell carcinoma. Epstein Barr virus was not detected in the oesophageal cancer.

4. Cytokeratin expression

- **Lam KY, Loke SL, Shen XC, Ma LT. Cytokeratin expression in non-neoplastic oesophageal epithelium and squamous cell carcinoma of the esophagus. *Virchows Arch A* 1995;426:345-49.**

The paper showed that the cytokeratin expression profile was different in normal and neoplastic oesophagus. The cytokeratin expression also varied with the differentiation of the oesophageal squamous cell carcinoma.

5. Tumour-related genes

- **Lam KY, Tsao SW, Zhang D, Law S, He D, Ma L, Wong J. Prevalence and predictive value of p53 mutation in patients with oesophageal squamous cell carcinomas: a prospective clinicopathological study and survival analysis of 70 patients. *Int J Cancer* 1997;74:212-19.**
- **Lam KY, Tin L, Ma L. c-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. *Eur J Surg Oncol* 1998;24:431-35.**
- **Lam KY, Law S, Lo T, Tung HM, Wong J. The clinicopathological significance of p21 and p53 expression in oesophageal squamous cell carcinoma: an analysis of 153 patients. *Am J Gastroenterol* 1999;94:2060-68.**
- **Hu YC, Lam KY, Tang JCO, Srivastava G. Mutational analysis of the PTEN/MMAC1 gene in primary oesophageal squamous cell carcinomas. *J Clin Pathol Mol Pathol* 1999;52:353-56.**
- **Chow V, Law S, Lam KY, Luk JM, Wong J. Telomerase activity in small cell oesophageal carcinoma. *Dis Esophagus* 2001;14:139-42.**

These papers highlighted the roles of p53, p21 and telomerase in the assessment of prognosis of patients with oesophageal cancers. c-erbB-2 (Her-2) mutation occurred in early stage of carcinogenesis. No PTEN mutation was found in oesophageal squamous cell carcinomas.

6. Gene mapping

- **Tang JCO, Lam KY, Law S, Wong J, Srivastava G. Detection of genetic alterations in oesophageal squamous cell carcinomas and adjacent normal epithelia by comparative DNA fingerprinting using inter-simple sequence repeat PCR. *Clin Cancer Res* 2001;7:1539-45.**
- **Hu YC, Lam KY, Law S, Wong J, Srivastava G. Identification of differentially expressed in oesophageal squamous cell carcinoma (ESCC) by cDNA expression array: overexpression of Fra-1, Neogenin, Id-1 and CDC25B genes in ESCC. *Clin Cancer Res* 2001;7:2213-21.**
- **Hu YC, Lam KY, Law S, Wong J, Srivastava G. Profiling of differentially expressed cancer-related genes in oesophageal squamous cell carcinoma (ESCC) using human cancer cDNA arrays: overexpression of oncogene MET correlates with tumour differentiation in ESCC. *Clin Cancer Res* 2001;7:3519-25.**
- **Kwong D, Lam A, Guan X, Law S, Tai A, Wong J, Sham J. Chromosomal aberrations in oesophageal squamous cell carcinoma among Chinese: gain of 12p predicts poor prognosis after surgery. *Hum Pathol* 2004;35:309-16.**

In these papers, genetic mutations in oesophageal squamous cell carcinomas were mapped by gene arrays and comparative genomic hybridization. The different newly discovered genetic alterations were analysed both in laboratory and in relationship with the prognosis of patients.

F. Author contributions

Statement making clear my contribution to all jointly authored publications

I contributed in all the selected 32 publications in the design, running of experiments and writing of the manuscripts. I am the first author in 20 of these publications I submitted. For the other 12 publications, I contributed significant amount of effort as a co-author (I act as the 2nd author in all except 3).

Alfred K. Y. Lam