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ORIGINAL ARTICLE

Cancer Biomarkers and Molecular Theranostics

ESK Ma, CLP Wong

Division of Molecular Pathology, Department of Pathology, Hong Kong Sanatorium and Hospital, 2 Village Road, Happy Valley, Hong Kong

ABSTRACT

Cancer biomarkers have evolved from assays based on proteins, hormones and enzymes to molecular assays based on DNA or RNA. These molecular cancer biomarkers have broad clinical applications in disease screening, diagnosis, classification, offering a prognosis, risk stratification, treatment selection and monitoring. The prevailing trend in oncology is to harness molecular biomarkers to predict treatment efficacy or toxicity in the individual patient, and thereby guide the choice of treatment. The term theranostics was recently coined to indicate this marriage between an individual's specific diagnosis and tailored therapy. Before therapeutics can be directly linked to diagnostics, the analytical validity, clinical validity, and clinical utility of the test in question should be carefully considered, not to mention the consequential ethical and financial implications. Recourse to predictive cancer markers with a view to targeted molecular therapy may have positive and negative connotations. The best known examples of positive predictors are the EGFR *gene mutation in lung cancer and the presence of* HER2 *gene amplification in breast cancer, whilst the* KRAS *gene mutation is a negative predictor in metastatic colorectal cancer. Development of companion diagnostics is now double fuelled by the ever-expanding repertoire of agents used for targeting; detection of* EML4-ALK *gene fusion for consideration of ALK inhibitor therapy in lung cancer being a recent example of this phenomenon. Apart from such predictive markers, pharmacogenomics is another important facet of cancer theranostics. For instance, thymidylate synthase expression or genotype is related to the response to 5-fluorouracil and related compounds. It is envisaged that in the future, emerging diagnostic tools — including the next generation of sequencing technology and array-based comparative genomic hybridisation applied to the cancer genome — will further advance personalised oncology.*

Key Words: *Breast neoplasms; Colorectal neoplasms, hereditary nonpolyposis; Lung neoplasms; Mutation/genetics; Tumor markers, biological*

中文摘要

腫瘤生物標記物及分子「治療診斷學」

馬紹鈞、黃利寶

腫瘤生物標記物的發展已經從蛋白質、賀爾蒙及酶演變至DNA或RNA的分子測定法。這些分子腫瘤生物標記物應用廣泛，可用作為對疾病的篩選、診斷、分類、預後評估、風險評級、選擇治療方法及監察。現今腫瘤學的趨勢是利用分子生物標記物來預測個別病人的治療效果或所產生的毒性，從而引導為病人選擇治療計劃。「治療診斷學」這詞正是用來表達將個人病情的診斷以及度身訂造的治療計劃合併的含義。在治療連繫診斷前，必需小心考慮測試本身的分析可信性、臨床有效性及應用；此外亦要考慮引起的道德及費用負擔問題。預測性腫瘤標記物在標靶分子治療中可分為陽性及陰性兩種。陽性預測因子的最佳例子如肺癌中的*EGFR*基因突變，或乳癌中的*HER2*基因擴增，這些基因都為標靶治療起了陽性預測作用。另一方面，*KRAS*基因突變在轉移性的結直腸癌中起了一個陰

Correspondence: *Dr Edmond S K Ma, Clinical Pathology Laboratory, 1/F Li Shu Fan Block, Hong Kong Sanatorium & Hospital, 2 Village Road, Happy Valley, Hong Kong.*
Tel: (852) 2835 8017 ; Fax : (852) 2835 8799; Email: eskma@hksh.com

性預測作用。不斷擴大的標靶治療藥物庫加倍催谷了配套檢測的發展，最新的例子是利用*EML4-ALK*融合基因的檢定作治療肺癌中ALK抑制劑治療的考慮。除了這些預測性標記物外，藥物基因組學是腫瘤治療診斷學的另一重要範疇，其中一個例子是胸腺嘧啶合成酶的表達水平或基因型與5-氟尿嘧啶及其合成物的反應有關。可以想像得到，新興的診斷工具（包括新一代的基因測序技術及應用在癌症基因組的基因芯片的比較基因組雜交技術）會進一步提升個人化腫瘤治療。

INTRODUCTION

Molecular cancer biomarker assays based on DNA and RNA testing are widely utilised in clinical oncology. These markers aid in disease diagnosis both in terms of confirming histological findings and offering a diagnostic label, even when conventional methods are inconclusive or inadequate. Notable examples include the molecular diagnosis of acute promyelocytic leukaemia, which is an oncological emergency. This involves reverse transcription–polymerase chain reaction (RT-PCR)[1] or fluorescence in-situ hybridisation (FISH),[2] whenever the morphology is atypical, and the differentiation of essential thrombocythaemia from reactive thrombocytosis through detection of the clonality marker JAK2 V617F mutation in the former condition.[3] Besides diagnosis, disease classification may be based on molecular markers, the first example of which is found in the World Health Organization classification of cytogenetically normal acute myeloid leukaemia (AML) based on the mutational status of *NPM1*[4] and *CEBPA*.[5] These gene mutations, together with the gene fusions resulting from chromosomal translocations, are powerful prognostic markers in AML. Risk stratification of patients can be achieved through a combination of several prognostic markers. For example, the combined use of interphase FISH markers and immunoglobulin heavy chain gene mutation status can identify subgroups at risk of chronic lymphocytic leukaemia.[6] What is most relevant to the practising oncologist is to directly use the molecular cancer biomarkers to guide therapy, and will be further elaborated on in the article. After treatment is administered, monitoring of the clinical response can be undertaken through the utilisation of molecular markers, which often allows greater analytical sensitivity or depth than is possible by conventional means. The most successful example of molecular monitoring prevails in chronic myeloid leukaemia, where international guidelines are available to define the milestones of optimal treatment response and standardisation of the testing method.[7] Finally, use of molecular markers in cancer screening is beginning to emerge, e.g. plasma EBV DNA is used to screen for nasopharyngeal cancer in our locality.[8]

Theranostics is a newly coined term that refers to the utilisation of diagnostic markers to direct therapeutic decisions, with the objective of individualising patient therapy.[9] These diagnostic markers are often molecular in nature and also termed predictive markers, because they predict either clinical outcome (prognosis) or treatment response. The latter predictive markers can be further categorised into positive or negative therapeutic predictors. Thus, epidermal growth factor receptor (*EGFR*) gene mutation in non–small-cell lung cancer (NSCLC) and human epidermal growth factor receptor-2 (*HER2*) gene amplification in breast cancer are positive. Whereas, the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene mutation in metastatic colorectal cancer (CRC) is classified as negative. Notably, before the predictive markers are put into routine diagnostic use, they have to be validated for both analytical performance (in terms of measurement accuracy) and clinical performance (in terms of distinguishing normal from the diseased). Even more desirable is the documentation of their clinical utility (i.e. evidence of survival benefit), but this may not always be available when the marker is first launched. Also to be considered are issues related to the patent for the diagnostic tests and the method of reimbursement, especially with respect to constraints due to tight health care budgets. Since the literature on the subject is expanding at a phenomenal pace and is widely disseminated over the internet, it is important for the relevant patient population be properly educated and counselled on the predictive markers to improve their uptake and avoid undue anxiety over test results.

PHARMACOGENOMICS

Apart from the predictive markers (related to tumour factors or drug targets), another important facet of personalised oncology is pharmacogenomics (host factors). There has been increasing utilisation of genetic polymorphisms, especially with respect to drug-metabolising enzymes that could predict treatment

efficacy or complications due to anti-cancer drugs.[10]

Most of the enzymes involved in drug metabolism are members of the cytochrome P450 superfamily; *CYP2D6* is one of the most widely studied. Tamoxifen, a selective estrogen receptor modulator, is standard treatment for patients with estrogen or progesterone receptor–positive breast cancer. Genetic variants of *CYP2D6* that result in reduced metabolism of tamoxifen to its active metabolites may lead to reduced drug efficacy, but the role of *CYP2D6* genotypes in predicting tamoxifen-associated clinical outcome remains unsettled and remains an area of intense investigation.[11]

The uridine diphosphate glucuronosyltransferase (UGT) superfamily of endoplasmic reticulum–bound enzymes is responsible for conjugating a glucuronic acid moiety to a variety of compounds to allow their elimination, whilst *UGT1A1* catalyses the glucuronidation of bilirubin for excretion in the bile. As irinotecan therapy for advanced CRC became more widely used, it was observed that patients with Gilbert's syndrome suffered from severe toxicity. An intermediary metabolite SN-38 of irinotecan shares the same glucuronidation pathway with bilirubin and its reduced clearance leads to more pronounced toxicity. The most common polymorphism associated with low activity of *UGT1A1* is the *28 variant with 7 (rather than 6) TA repeats at the gene promoter, in which case irinotecan dose reduction is recommended in patients homozygous for that allele.[12]

Frequently, 5-fluorouracil (5-FU) is used together with irinotecan in advanced CRC and acts by inhibition of thymidylate synthase (TS). This leads to depletion of the thymidine in the cell, which shuts off DNA synthesis and triggers apoptosis and other cell death processes. A substantial body of evidence has accumulated over the past few years, showing that TS expression varies considerably among tumours and the clinical response to 5-FU and related compounds is inversely related to TS expression. Low TS expression is associated with significantly higher response rates and a superior median survival as compared to high TS expression tumours. Polymorphisms within the *TYMS* gene regulate TS expression and have even been found to exert an effect on the response to 5-FU–based chemotherapy.[13] The significant polymorphisms include either double (*2R*) or triple (*3R*) tandem repeats of 28-bp sequences at the 5'-UTR, a G→C substitution at the second repeat of *3R* allele, and a 6-bp deletion at the 3'-UTR of the gene. The *2R*, *3RC* and *-6* alleles are associated with low levels of TS expression. Therefore the high expression genotypes are *2R/3RG*, *3RC/3RG*, *3RG/3RG* plus *+6/+6* or *+6/-6*, whereas the low expression genotypes are *2R/2R*, *2R/3RC*, *3RC/3RC* plus *-6/-6*. Such *TYMS* genotyping may obviate the methodological drawbacks of intra-tumoural TS mRNA and protein expression studies.[14] However, germline polymorphism is only a predictor of expression without taking into account acquired changes in the tumour and the interaction of different polymorphisms may be more complicated than expected.

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that inactivates thiopurine drugs such as 6-mercaptopurine and azathioprine through methylation, before their conversion into 6-thioguanine nucleotides, the cytotoxic metabolites incorporated into DNA. Variability of TPMT enzyme activity is attributable to polymorphisms of the *TPMT* gene. The most common alleles (*TPMT*2*, *TPMT*3A* and *TPMT*3C*), account for 95% of TPMT deficiency. Genotyping is therefore a convenient method for predicting TPMT enzyme activity before treatment with thiopurines.[15] Low TPMT activity puts the patient at risk of developing toxicity, whereas high activity indicates a need to use a higher dose.

In addition to pharmacogenomic markers, protein and mRNA expression levels of ERCC1 and RRM1 appear to influence the response to cisplatin and gemcitabine respectively in NSCLC, and are therefore determinants of survival after surgical treatment of early-stage disease.[16]

HER2 AMPLIFICATION IN BREAST CANCER

The *HER2* gene is amplified in approximately 18 to 20% of breast cancers and is the primary mechanism of HER2 protein over-expression on the tumour cells. The HER2 status is associated with clinical outcome in breast cancer. HER2 positivity predicts an inferior prognosis (i.e. higher recurrence rate and mortality) in newly diagnosed breast cancer patients not in receipt of adjuvant systemic therapy. More importantly, the monoclonal antibody trastuzumab that targets the receptor is highly effective in HER2-positive breast cancer patients, in both the metastatic and adjuvant setting. Recently the small molecule tyrosine kinase inhibitor (TKI) lapatinib was reported to improve clinical outcome in patients with advanced breast cancer when added to capecitabine.[17] Overall these results

show the usefulness of HER2 testing in therapeutic decision making for breast cancer patients.

Detection of HER2 status in breast cancer is routinely performed by immunohistochemistry (IHC) for protein over-expression and FISH for gene amplification. Recommendations for the testing algorithm, definition of positivity and quality assurance issues were recently issued jointly by the American Society of Clincial Oncology and College of American Pathologists.[18] A positive HER2 result was defined as IHC 3+ (i.e. uniform, intense membrane staining of >30% of invasive tumour cells) or FISH+ (i.e. >6 average *HER2* gene copies per nucleus or a ratio of *HER2* gene signals to chromosome 17 signals of >2.2). An equivocal HER is said to occur in face of IHC 2+, there are between 4 and 6 *HER2* gene copies per nucleus or the FISH ratio is 1.8 to 2.2. A negative HER2 result is inferred from an IHC of 0 or 1+, a FISH result of <4 copies or FISH ratio of <1.8. Studies have shown that only patients with IHC 3+ staining and / or *HER2* gene amplification by FISH benefit from trastuzumab,[19] and that only 20 to 25% of the IHC 2+ tumours show gene amplification when tested by FISH. Therefore the algorithm for IHC is to reflect to FISH for 2+ cases only, noting that when an equivocal result is encountered, patients with a *HER2*/CEP17 ratio of ≥2 are eligible for trastuzumab. The algorithm for FISH in the event of an equivocal result involves counting additional cells, repeat testing, or performing the IHC testing (or reviewing the IHC result if already available).

The advantage of using an internal control for the copy number of chromosome 17 is to distinguish between *HER2* gene amplification and polysomy 17.[20] The presence of polysomy 17 may complicate the interpretation of HER2 testing and is a major cause of equivocal results (Figure 1). Polysomy 17 per se does not significantly increase mRNA levels but may increase HER2 protein expression (used in the IHC 2+ range). In contrast to *HER2* gene amplification, polysomy 17 is not associated with high-grade tumour, hormone receptor negativity and reduced disease-free survival, and therefore biologically it resembles HER2 negativity. In the rare event of polysomy 17 found in association with IHC 3+, the ploidy level is usually high and the indication for trastuzumab is established by the IHC result.[21]

HER2 over-expression is also detectable in gastric cancer, and an early study showed an incidence of 12.2% in gastric and 24% in gastroesophageal adenocarcinoma.[22] The recently completed ToGA trial reported that trastuzaumab in combination with chemotherapy may be considered a new standard option for patients with HER2-positive advanced gastric cancer.[23] The HER2 testing in gastric cancer differs from that in breast cancer in 2 aspects.[24] First, owing to their structure, the basolateral membranous immunoreactivity of the glandular cells may result in incomplete membranous staining (in contrast to complete and uniform staining in breast cancer). Second, compared to breast cancer, in gastric lesions a higher rate of heterogeneity may necessitate the examination of more tumour areas.

Emerging technologies such as silver in-situ hybridisation (SISH) are being developed for the purpose of HER2 testing and show excellent concordance with FISH in breast cancer.[25] The interpretation of SISH results is most reproducible using the *HER2*/CEP17 ratio. However separate slides are needed for detection of *HER2* gene copy number and chromosome 17 signals. To circumvent the issue, a dual-colour dual-hapten in-situ hybridisation (dual ISH) system was recently developed in which the *HER2* gene signal was detected by SISH whilst the chromosome 17 copy number was detected by chromogenic red ISH on the same slide.[26] The advantages of these new ISH systems include automated testing, facilitation of reflex testing after IHC on the same machine, direct identification of the tumour cells under bright-field microscopy, and amenability for later review.

A related gene locus which is adjacent to and slightly telomeric to the *HER2* gene is the topoisomerase II-a (*TOP2A*) gene, the amplification or deletion of which is often linked with *HER2* gene amplification and increased responsiveness to anthracycline-containing chemotherapy regimens.[27] The *TOP2A* status can be detected on its own, and paired with chromosome 17 copy number controls by dual-colour FISH, or together with *HER2* status (by triple-colour FISH).

EGFR GENE MUTATION IN NON–SMALL-CELL LUNG CANCER

Activating mutations of the *EGFR* gene are highly correlated with treatment responsiveness to gefitinib and erlotinib, both of which are small molecule TKIs targeting *EGFR*. These mutations occur at the tyrosine kinase domain of the *EGFR* gene and preferentially involve a subset of lung cancers characterised by

female sex, non-smoker, adenocarcinoma histology, and East Asian ethnicity. *EGFR* mutations involve the adenosine triphosphate–binding pocket of the tyrosine kinase receptor domain, clustering at exons 18 to 21 of the *EGFR* gene,[28] and in the form of in-frame deletions, in-frame insertions/duplications and point mutations. Two hotspots namely deletion at exon 19 and the point mutation L858R at exon 21 constitute the majority of mutations. These mutations increase the kinase activity of EGFR, leading to the heightened activation of downstream pathways that favour cell survival on the one hand, but also opens up an avenue for inhibition through TKI therapy on the other. Patients with NSCLC having exon 19 deletions seem to respond better to gefitinb and erlotinib than those with L858R mutations.[29] A few mutations — notably D761Y, S768I, T790M, and insertions at exon 20 — are resistant to TKI therapy.[30,31] Two recent randomised clinical trials IPASS[32] and SATURN[33] have confirmed that the *EGFR* mutation status is a strong predictive biomarker of clinical benefit from TKI therapy, but the same cannot be said for *EGFR* gene copy number by FISH or EGFR protein expression by IHC.

Besides serving as a cancer biomarker to predict TKI response in NSCLC, the detection of different *EGFR* gene mutations in multiple lung cancers enables the distinction between synchronous primaries from intrapulmonary metastases,[34] and metachronous primaries from disease recurrence.

The detection of *EGFR* mutations was performed

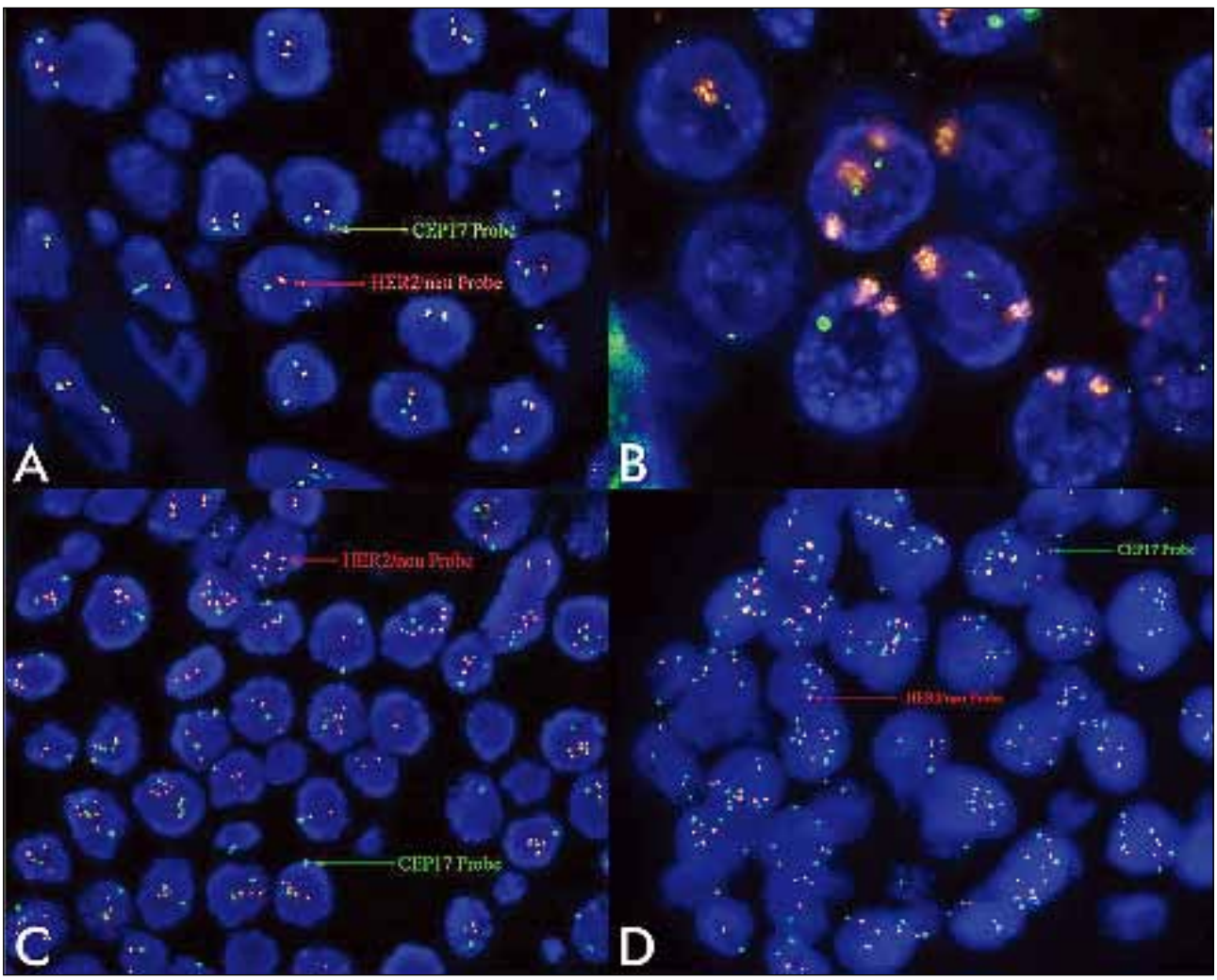

Figure 1. Detection of *HER2* gene amplification by dual-colour interphase fluorescence in-situ hybridisation (PathVysion, Abbott Molecular). The orange probe hybridises to the *HER2* gene whereas the green probe hybridises to the α-satellite region of the chromosome 17 centromere (CEP17) and enumerates its copy number. (a) HER2 negative with *HER2*/CEP17 ratio of 0.96 and IHC 2+; (b) HER2 amplification in the form of homogenous staining region or micro-clusters; (c) low-level HER2 amplification with *HER2*/CEP17 ratio of 2.47 in a case with IHC 2+ status; and (d) polysomy 17 with *HER2*/CEP17 ratio of 1.1 and IHC 2+.

at Hong Kong Sanatorium and Hospital by PCR, followed by Sanger sequencing on DNA extracted on micro-dissected paraffin tissue to enrich tumour cells.[35] A review of 481 consecutive samples accrued from September 2005 to April 2009 at our laboratory showed detectable *EGFR* mutations in 210 samples (44%) overall. Among the positive samples, exon 19 deletions accounted for 86 cases (41%) in which ELREA deletion accounted for 62 cases (30%) and exon 21 L858R mutations accounted for 82 cases (39%). These two mutation hotspots accounted for 80% of cases, which was in accordance with international literature. Rare insertion mutants were detected at exon 20 and although some were unreported, they were most probably associated with primary or de-novo drug resistance, especially those at position D770. These should be distinguished from secondary or acquired resistance conferred by T790M that was usually detected in association with a hotspot drug sensitive mutation, as seen in one of our cases. Double mutations were detected in 22 cases (10%) and usually involved at least one TKI-sensitive mutant. Unexpectedly, 3 cases showed a combination of a TKI-sensitive hotspot mutation and a known TKI resistance exon 20 S768I mutation, in whom documentation of the TKI response would be of interest. Finally, although *EGFR* and *KRAS* mutations should be mutually exclusive,[36] one sample showed concurrent presence of both, which might reflect tumour heterogeneity.

Reflex *KRAS* mutation detection was performed only in samples tested negative for *EGFR* gene mutation, unless a specific request was made. Among the 271 (56%) *EGFR* mutation–negative cases, *KRAS* mutations were detected in 27; all were hotspot mutations at exon 2 (Gly12Phe = 1, Gly12Cys = 7, Gly12Asp = 1, Gly12Ala = 4, Gly12Val = 12, and Gly13Asp = 2). *KRAS* mutations accounted for 10% of all *EGFR* negatives and 5.6% of all cases. Overall 244 cases (51%) of samples were negative for both *EGFR* and *KRAS* gene mutations.

EML4-ALK GENE FUSION IN NON–SMALL-CELL LUNG CANCER

First reported in 2007, this new molecular aberration in NSCLC occurs as a result of a small inversion within chromosome 2p that leads to the formation of a fusion gene that comprises portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) upstream and the anaplastic lymphoma kinase (*ALK*) downstream.[37] The fusion gene, initially reported at a frequency of 3 to 5% in NSCLCs, was oncogenic and represented a novel molecular target. The patients tended to be younger, more likely to be male, never or light smokers, and tested negative for *EGFR* and *KRAS* gene mutations.[38] *EML4-ALK* fusion may be detected by a dual-colour split-apart FISH probe that targets the *ALK* gene (Abbott Molecular, Abbott Park, IL).[38] While the typical FISH signal pattern in a positive cell emits one fusion yellow signal, one orange signal and one green signal, atypical signal pattern in the form of one fusion yellow signal and one orange is also recognised as a result of deletion of the 5' portion of the rearranged *ALK* gene (Figure 2). A dual-colour dual fusion probe system was also developed but since both

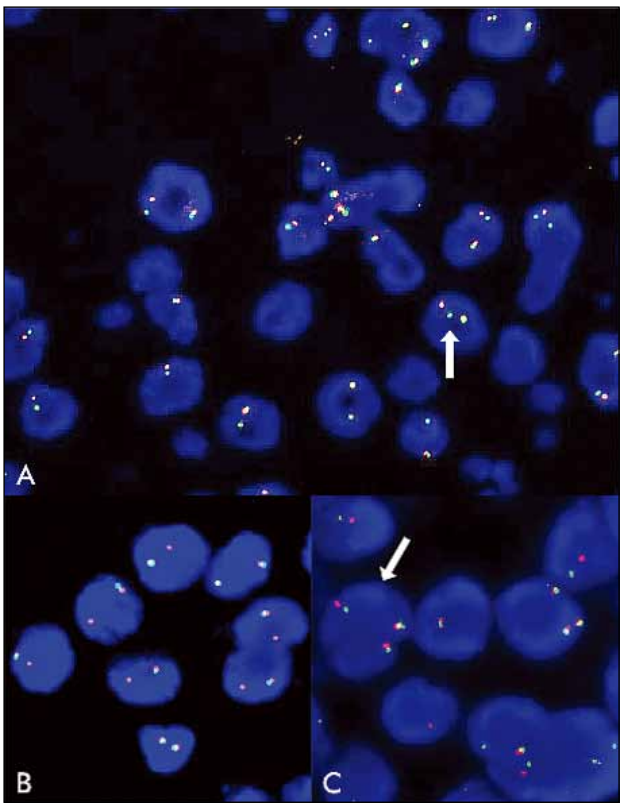

Figure 2. Detection of *ALK* gene rearrangement by dual-colour interphase fluorescence in-situ hybridisation. (a) ALK dual-colour split apart probe (Abbott Molecular) showing one yellow fusion signal of wild-type allele, and split orange signal and green signal indicating *ALK* translocation or rearrangement (arrow). (b) ALK dual-colour split apart probe (Abbott Molecular), showing atypical signal pattern with one yellow fusion signal of wild-type allele and one residual orange signal. The green probe targeting the 5'-portion of the rearranged *ALK* allele is deleted. (c) Dual colour dual fusion *EML4-ALK* probe (GSP, Japan) showing two fusion yellow signals, one green signal targeting the wild-type *EML4* gene and one red signal targeting the wild-type *ALK* gene. Since both genes that are involved in the fusion are located on the same chromosome arm, the distinction between wild type and fusion signals may be more challenging than inter-chromosomal translocations.

genes involved in the fusion were originally located on the same chromosome arm, the distinction between wild type and fusion signals may be more challenging than for inter-chromosomal translocations. The fusion transcript can be detected by RT-PCR, and a multiplex method is reported that allows the characterisation of various isoforms of the fusion transcript.[39] Finally, the utilisation of IHC for cytoplasmic expression of ALK protein in the detection of *EML4-ALK* fusion is also under investigation. Patients who harbour this gene fusion are not expected to derive clinical benefit from *EGFR* TKI and are candidates for trials of *ALK*-targeted therapy (e.g. crizotinib).

KRAS GENE MUTATION IN COLORECTAL CANCER

The benefit of anti-EGFR monoclonal antibody cetuximab in advanced CRC is limited to patients with wild-type *KRAS* tumors but not in those harbouring mutations in codons 12 or 13 of the *KRAS* gene.[40] There is, however, a lack of validated testing methods and standardised protocols for the detection of *KRAS* gene mutations. Few studies have systematically compared the sensitivity, specificity, and concordance between different diagnostic methods. Similar to *EGFR* mutation testing, the methodologies currently in use can be categorised as allele-specific PCR assays that cover specific mutations and direct nucleotide sequencing that covers all variations that may be present. This has to be balanced against the higher analytical sensitivity of mutation detection for the former assays (*vide infra*). For example, while the TheraScreen (DxS, Manchester, UK) allows the detection of a *KRAS* mutant clone size down to 1%, only 7 codon 12 and 13 mutations are targeted by the TheraScreen assay; rarer ones are not covered. Moreover, mutations at exon 3 and other positions of exon 2 (apart from codons 12 and 13) are also not detected. The higher detection sensitivity of allele-specific PCR assays obviates the need for micro-dissection and is simpler to perform when compared with direct sequencing, but are considerably more expensive.

At Hong Kong Sanatorium and Hospital, we retrospectively analysed 210 consecutive samples from patients with CRC submitted for *KRAS* mutation detection from May 2008 to April 2009 (Table 1). DNA was extracted from formalin-fixed paraffin-embedded tissue after micro-dissection to select areas with at least 30% tumour cells by a histopathologist. Detection of *KRAS* mutations at exons 2 and 3 was performed by PCR sequencing. Bidirectional sequencing was performed. Abnormal results were repeated for confirmation to exclude PCR artefacts. *KRAS* gene mutations at codons 12 and 13 were identified in 76 samples (36%). Double mutations were seen in 3 patients. Of note, 5 (7%) of 76 samples harbouring rare mutations at codons 12 and 13, namely Gly12Phe (n = 1), Gly13Arg (n = 2) and Gly13Cys (n = 2) respectively, would have escaped detection by TheraScreen. Finally,

Table 1. The results of *KRAS* gene mutation detection in 210 patients.

Codon	Nucleotide change	Amino acid change	No. of cases
12	c.34_35delinsTT	Gly12Phe*	1
12	c.34G→A	Gly12Ser	2
12	c.34G→T	Gly12Cys	4
12	c.35G→A	Gly12Asp	28
12	c.35G→C	Gly12Ala	8
12	c.35G→T	Gly12Val	16
13	c.37G→C	Gly13Arg*	2
13	c.37G→T	Gly13Cys*	1
13	c.38G→A	Gly13Asp	11
15	c.43G→A	Gly15Ser	1
19	c.57G→C	Leu19Phe	1
22	c.64C→A	Gln22Lys	1
29	c.85G→A	Val29Met	1
33	c.99T→A	Asp33Glu	1
37	c.109G→A	Glu37Lys	1
61	c.183A→T	Glu61His	1
8 & 12	c.22G→T & c.35G→T	Val8Leu & Gly12Val	1
12 & 63	c.35G→T & c.187G→A	Gly12Val & Glu63Lys	1
13 & 60	c.37G→T & c.179G→A	Gly13Cys* & Gly60Asp	1
	No KRAS mutation detected in exons 2 & 3		127
	Total No. of cases		210

* *KRAS* gene mutations at codons 12 and 13 not detected by TheraScreen.

Table 2. Comparison of *KRAS* gene mutations in non–small-cell lung cancer (NSCLC) and colorectal cancer (CRC)*

	Transversion (purine for pyrimidine or vice versa nucleotide substitution)	Transition (purine for purine or pyrimidine for pyrimidine nucleotide substitution)
NSCLC (n = 77)	53	24
CRC (n = 102)	42	60

* Fisher's exact test: p = 0.0003.

10 mutations (5%) of uncertain clinical significance were detected outside codons 12 and 13.

The superior analytical sensitivity of the allele-specific PCR assays should be balanced against the inability of these assays to detect rare but significant mutations at codons 12 and 13 due to the inherent specificity of target selection. In our patient cohort, 6.6% of mutations that occurred at codons 12 and 13 would not be detected by TheraScreen. Direct sequencing analysis can detect all mutations but at a lower analytical sensitivity. Sensitivity may be improved however, by micro-dissection to enrich tumour cell contents or newer approaches such as high-resolution melting studies which are superior to conventional Sanger sequencing.[41] Moreover, a recommendation on the reporting of mutations other than codons 12 and 13 is needed for the sake of laboratories that employ direct sequencing analysis. In our laboratory, this scenario was encountered in 4.8% of all cases and included an activating mutation at codon 61, which appears to be an uncommon but recurrent mutation of potential clinical significance.

Is There Any Difference between the *KRAS* Mutations in Colorectal Cancer and Non–small-cell Lung Cancer?

KRAS gene mutations occur in both CRC and NSCLC albeit at different frequencies. Interestingly, we compared the type of mutation in terms of nucleotide change between the *KRAS* gene mutations in the 2 cancers and found that transversions were over-represented in NSCLC and transitions were over-represented in CRC, of which the difference achieved statistical significance (Table 2). This difference was probably due to differing pathogenesis of the cancers but the clinical or therapeutic implication is hitherto unknown.

CONCLUSION

The era of molecular theranostics in cancer paves the way for a paradigm shift in pathology, from purely providing a diagnosis and investigating disease mechanisms, to directly ensuring appropriate treatment. With an increasing role to play,[42] the pathologist should keep abreast of recent developments in companion diagnostics of targeted therapy, since clinical practice is expected to move ahead rapidly and new cancer biomarkers once validated will replace existing ones. With respect to the cancer genome, it is envisaged that emerging, next-generation, diagnostic sequencing technology to detect mutations as well as array-based comparative genomic hybridisation (to interrogate copy number variations) will further advance personalised oncology.[43]

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