KRAS Mutation Testing in Advanced Colorectal Cancers

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KRAS is an oncogene located on the short arm of chromosome 12 (12p12.1). The gene is a member of the Ras family of small guanine nucleotide–binding proteins, first identified as a cellular homolog of a transforming gene in the Kirsten rat sarcoma virus.[1] Activating mutations in KRAS have been noted in a variety of human cancers, including carcinomas of the pancreas, lung (non-small cell lung cancer), and colorectum (Table 1). KRAS mutations are frequently found in exon 1 (codons 12 and 13) and exon 2 (codon 61).[2] Mutations in KRAS codons 12 and 13 have been associated with lack of response to EGFR-targeted therapies in patients with colorectal cancer (CRC) (Table 2).[3, 4]

In 2009, The American Society of Clinical Oncology (ASCO) issued their first provisional clinical opinion recommending that all patients with CRC who are candidates for anti-EGFR monoclonal antibody therapy should undergo testing for KRAS mutations and that patients with KRAS should not receive anti-EGFR monoclonal antibody therapy as part of their treatment.[5] Common 7 somatic mutations in KRAS codons 12 and 13 have been recommended for evaluation of KRAS gene mutation in CRC (Table 3).[3-5]

Table 1: Frequency of KRAS Mutations in Human Cancers[1]

Tumor type	Frequency (%)
Pancreas	59
Biliary tract	32
Large intestine	32
Small intestine	20
Gastrointestinal tract (site indeterminate)	19
Lung	18
Ovary	15
Thymus	15
Endometrium	14

Table 2: Impact of KRAS Mutations in Patients with Metastatic CRC Treated with EGFR

Therapy	Response Rate		Median Survival	
	Positive for KRAS Mut	Negative for KRAS Mut	Positive for KRAS Mut	Negative for KRAS Mut
Cetuximab	0/36 = 0%*	34/78 = 44%*	9 wks (PFS)	32 wks (PFS)
Panitumumab	0/84 = 0%**	21/124 =17%**	7 wks (PFS)	12 wks (PFS)

Mut = mutation, PFS = progression-free survival, wks = weeks
** Response rate includes partial and complete responders.*
*** Response rate includes only partial responders.*

Table 3: Common 7 KRAS mutations in exon 1 (codons 12 and 13) in CRC

Codon 12	nt34G>A	Gly12Ser	G12S
	nt34G>C	Gly12Arg	G12R
	nt34G>T	Gly12Cys	G12C
	nt35G>A	Gly12Asp	G12D
	nt35G>C	Gly12Ala	G12A
	nt35G>T	Gly12Val	G12V
Codon 13	nt38G>A	Gly13Asp	G13D

Table 4: Comparison of Methods Used for KRAS Mutation Testing [2, 6]

	Sensitivity,% of mutant alleles	Closed PCR system	Closed PCR system	Labor time	Turnover time*, hours
Direct (Sanger) sequencing	20	No	++	+++	5
Pyrosequencing	5-10	No	++	++	3.5
Real-Time PCR (melting-curve analysis	5-10	Yes	+	+	2.5
DxS KRAS mutation detectio kit (allele-specific real-time PCR)	1	Yes	+++++	++	2.5

KRAS Testing in the Context of Anti-EGFR Therapy for Colorectal Cancer

KRAS plays a pivotal role in the EGFR signaling network. When epidermal growth factor occupies the EGFR, it activates a signaling pathway cascade through the downstream effectors of the mitogenactivated protein kinases (MAPK) pathway. KRAS is one of the effectors (in addition to BRAF, ERK, and MAPK) which influence cellular proliferation, adhesion, angiogenesis, migration, and survival1,[6] Blocking EGFR with monoclonal antibodies (cetuximab or panitumumab) inhibits all downstream effects of the receptor. However, if the signaling pathways are activated independent of EGFR, as happens when the KRAS gene is mutated, these anti-EGFR agents become ineffective.[1, 6] Mutation of other genes in the downstream pathway such as BRAF could result in the same effect.[6]

KRAS Mutation Testing Methodologies

Detection of KRAS mutations in colorectal cancer tissue can be done by several molecular methods. Commonly used techniques can be divided in 2 main categories: DNA sequencing and real-time PCR (polymerase chain reaction).

DNA Sequencing Direct (Sanger) sequencing and pyrosequencing can be used for KRAS mutation testing. While the former has been considered a gold standard, the technique is time-consuming and the sensitivity is rather low (20% of mutant alleles required for detection) (Table 4). Pyrosequencing (Figure1) is faster than Sanger, with a lesser amount of mutant alleles required for mutation detecting.[6]

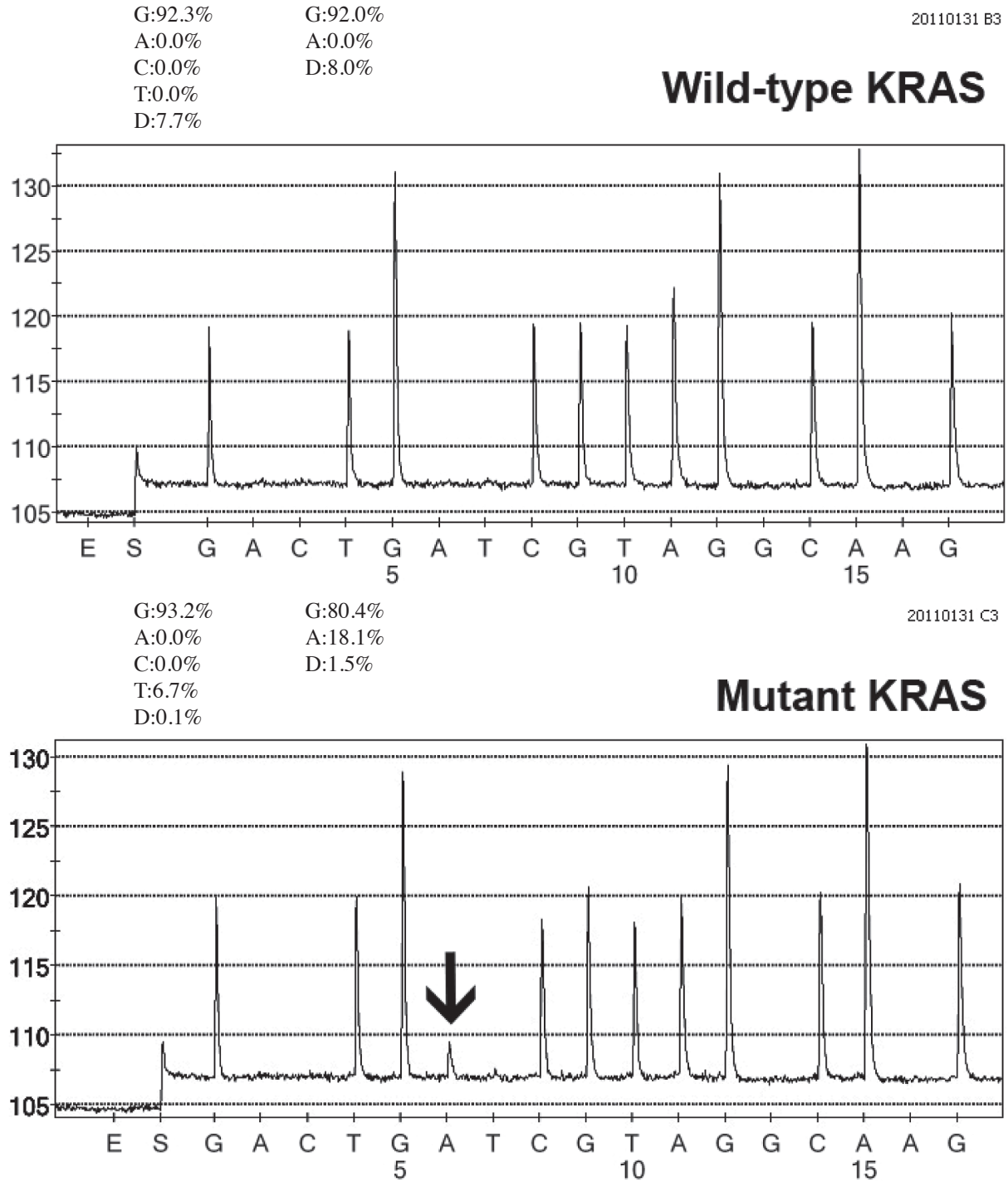
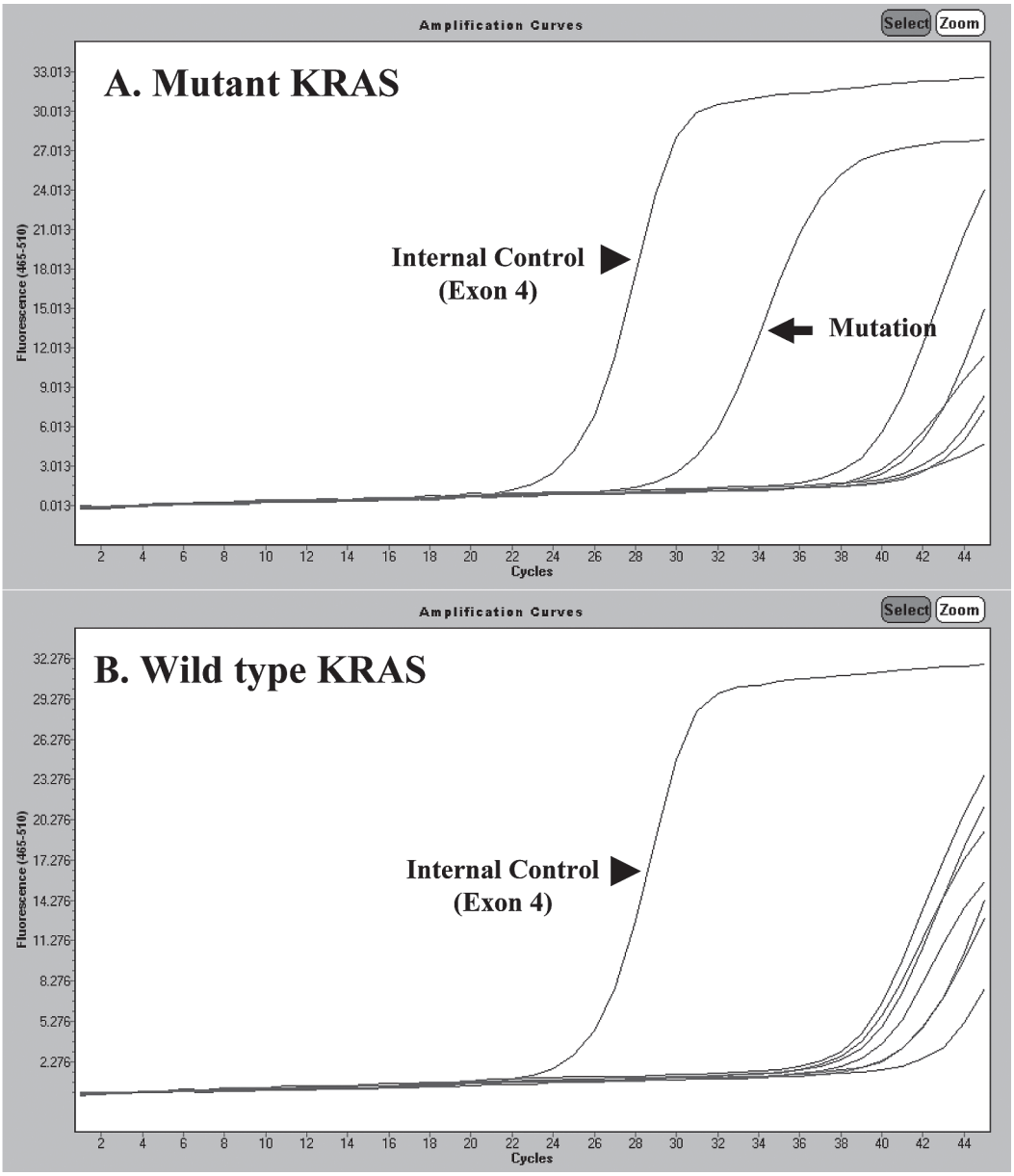


Figure 1: KRAS testing using pyrosequencing. *Pyrograms demonstrate wild-type KRAS gene (upper panel) and mutant KRAS gene (lower panel). Note a mutant "A" peak (arrow) indicating nt38 G>A (Gly13Asp, G13D) at codon 13, exon 1 of the KRAS gene. (Courtesy of Dr Chupong Ittiwut, PhD, Chulalongkorn GenePRO Center, Faculty of Medicine, Chulalongkorn University)*



C. Sample Result Table

Sample Name	Control Ct	Delta Ct	Flags / Warnings	Mutation Status
A	24.61	5.84	-	Positive 12ASP
B	25.34	-	-	Negative

Figure 2: KRAS testing using allele-specific real-time PCR (The DxS TheraScreen KRAS Mutation Kit). (A) *Positive for KRAS mutation. Note the second curve (arrow) representing the amplification of a KRAS mutation. (B) Negative for KRAS mutation. Note the curve at cycle 24 (arrowhead) served as internal control (exon 4 of the KRAS gene). (C) The DxS TheraScreen KRAS Mutation Kit analysis report using the LightCycler® Adapt Software v1.1 (Roche Diagnostics, Penzberg, Germany). (Courtesy of Dr. Alisa Junpee, PhD, Bio Molecular and Pathology Laboratory, National Healthcare Systems CO., Ltd)*

Real-Time PCR The presence of a KRAS mutation can be detected either by allele-specific real-time PCR amplification (Figure 2) or by post-PCR fluorescent melting-curve analysis. Both techniques are closed PCR system, thus reducing risk of contamination. The former is available as a commercial kit, and it is estimated to be able to detect only 1% of mutant allele.[6] However, the reagent cost is very high, and the technique requires more tissue for analysis as compared with other methods.[2, 6] The melting-curve analysis has the similar sensitivity as does the pyrosequencing, but distinguishing among mutation types can occasionally be problematic.[6]

KRAS Mutation in Colon Cancers in Thai Patients.

115 and 153 colon cancer specimens from Thai patients have been tested for KRAS mutations at Bio Molecular and Pathology Laboratory, National Healthcare Systems CO., Ltd by allele-specific real-time PCR (TheraScreen kit, DxS Ltd, Manchester, UK) and Chulalongkorn GenePRO Center (pyrosequencing), respectively (data kindly provided by Drs Chupong Ittiwut, PhD, and Alisa Junpee, PhD). 31.3% (36/115) and 37.9% (58/153) of cases were found to carry KRAS gene mutation, total mutant KRAS cases = 37.3% (94/268). Gly12Asp (G12D), Gly12Val (G12V), and Gly13Asp (G13D) are among the commonest KRAS mutation identified in both centers. The overall percentage of KRAS mutation found in our Thai patients is within the range (30-40%) previously described in the literature.[6]

Conclusion

The most challengers in oncology is that of patient selection for therapy with molecularly targeted agents. Kras are important determinants of response or resistance to anti EFGR antibodies.

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