QUALITATIVE AND QUANTITATIVE STUDIES ON DETECTION OF MICROMETASTASES IN COLORECTAL CANCER

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Papers:

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2. **Manuel ST, Kong SL, Adrian PKL and Koay ESC.** Intrinsic variability in the detection of micrometastases in lymph nodes for re-staging of colorectal cancer: Effect of individual markers and tissue sample. *Eur J Cancer* 2003; 39(9): 1234-1241.

Abstracts:

1. **Kong SL, Manuel ST, Adrian PKL and Koay ESC.** Quantitative studies on detection of micrometastases in colorectal cancer. *Abstract Book, 7th NUS-NUH Annual Scientific Meeting, 2nd-3rd, October 2003 Singapore*, in press.

2. **Kong SL, Manuel ST, Adrian PKL and Koay ESC.** Detection of nodal micrometastases in colorectal cancer using rapid quantitative real-time polymerase chain reaction. *Molecular Target For Cancer Therapy: Second Biennial Meeting, 11-15th, October 2002, USA, p18.*

3. **Kong SL, Manuel ST, Adrian PKL and Koay ESC.** Intrinsic variability in the detection of micrometastases in lymph nodes for re-staging of colorectal cancer: Effect of individual markers and tissue sample. *Abstract Book, 1st National Health Group Scientific Congress, 17-18th,August 2002, Singapore, p214.*

4. **Kong SL, Manuel ST, Adrian PKL and Koay ESC.** Molecular detection of micrometastases in fresh frozen and paraffin-embedded lymph nodes for re-staging of colorectal cancer using CEA, CK-20 and GCC as biomarkers. *Abstract Book, 6th NUS-NUH Annual Scientific Meeting, 16-17th, August 2002, Singapore, p89.*

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Abbreviations

Most of the abbreviations used are standard. However, attention is drawn to the followings:

Original terminology	Abbreviation
Ammonium persulphate	APS
and associates	*et. al.*
Base pair	bp
Carbon Dioxide	CO_2
Centrifugal force	*g*
Complementary Deoxyribonucleic Acid	cDNA
Crossing Point	Ct
Dalton	Da
Degree Centigrade	°C
Diethyl Pyrocarbonate	DEPC
Deoxyribonucleic Acid	DNA
Deoxyribonucleoside Triphosphates	dNTPs
Ethidium bromide	EtBr
Ethylenediaminetetraacetic acid	EDTA
Forward	F
Hanks balanced salt solution	HBSS
High Performance Liquid Chromatography	HPLC
Hour	h
Kilo	k
Magnesium Chloride	$MgCl_2$
Mean Square Error	MSE
Messenger Ribonucleic Acid	mRNA
Microgram	µg
Micromole	µmol
Micromole/litre	µM
Millilitre	mL
Minute	min
Not Available	NA
N, N, N', N'- Tetramethylethylenediamine	TEMED
N-(2-Hydroxylethyl) piperazine-N'-(2-ethanesulfonic acid)	HEPES
Nanogram	ng
Optical density	OD
Peripheral Mononuclear	PBMN
Polymerase Chain Reaction	PCR
Probability	p
Real-time Polymerase Chain Reaction	R-PCR
Regression	r

Reverse	R
Reverse Transcriptase-Polymerase Chain Reaction	RT-PCR
Room Temperature	RT
Second	s
Standard deviation	SD
Standard error	SE
Steriled	sd
Tris-Borate-EDTA	TBE
Ultraviolet	UV
Unit	U
Versus	vs
Volt	V

Summary

Purpose: The tumor spread and the radicality of surgical resection based on the histopathological evaluation are the most important facts in a patient's prognosis. Due to the early dissemination of tumor, many Dukes' B patients die from recurrence despite of curative tumor resection. We thus aim to develop a molecular approach to accurately assess the spread of submicroscopic nodal metastases in colorectal cancer (CRC). We investigated (a) the overexpression of carcinoembryonic antigen (CEA), cytokeratin 20 (CK20) and guanylyl cyclase c (GCC) in CRC, (b) the comparison between the results by qualitative and quantitative studies using conventional polymerase chain reaction (PCR) and real-time PCR (R-PCR) respectively, and (c) the effects of these analyses in the final re-staging of Dukes' B CRC.

Experimental Design: A total of 175 frozen lymph nodes (FT) and 158 formalin-fixed, paraffin-embedded lymph nodes (PET) from 28 CRC cases were studied. mRNA extractions from FT, PET and cell line followed by cDNA synthesis with RT-PCR were performed. CEA, CK20 and GCC-specific qualitative conventional PCR and quantitative R-PCR were carried out on the mRNA transcripts, using the gel-based electrophoresis and LightCycler® (LC) technology with SYBR Green I chemistry respectively. A separate PCR run for housekeeping gene is carried out and lymph nodes (LN) with no amplification were excluded from the data base, to eliminate false negative results.

Results: Our study demonstrated successful RNA extraction from 94.3% of FT and 70.7% of PET. The qualitative conventional PCR results indicated 90.9% morphologic Dukes' B CRC cases had detectable CEA or CK20 while 54.5% Dukes' B had detectable GCC. Higher sensitivity achieved in R-PCR methods allowed detection of low expression level of CEA and CK20 in our Dukes' A CRC cases and we arbitrarily considered positive any value above the quantification of the highest Dukes' A LN to distinguish between baseline constitutional expression and cancer-specific expression to prevent false positive results. Our quantitative R-PCR results indicated 63.6% morphologic Dukes' B cases had detectable CEA while 45.6% Dukes' B had detectable CK20 or GCC marker. In general, the differences of re-staged cases when comparing FT and PET sample with different tumor markers were marked.

Conclusions: Our results indicated a high incidence (>45%) of detecting micrometastases in histologically negative LN at the molecular level. There is discordance in the positivity of tumor markers in different tissue types (FT versus PET) and different methodology (gel-based qualitative studies versus LC-based quantitative studies). There is a considerable possibility that micrometastases may exist in histologically negative lymph nodes. However, their detection by RT-PCR coupled to gel electrophoresis was limited by the lack of reproducibility. We suggest LC-based quantitative studies is a better technology, which allows rapid and highly sensitive detection of micrometastases with minimum risk of contamination. Sensitivity of a single tumor marker in one tissue type (either in FT or PET) applied

by other investigators for the detection of micrometastases may be inadequate due to the heterogeneous composition of tumors. Multiple tumor markers are required to precisely predict the metastatic potential of Dukes' B CRC cases.