LETTER TO THE EDITOR

Frequency of Genetic Alterations Observed in Cell Cycle Regulatory Proteins and Microsatellite Instability in Gallbladder Adenocarcinoma: A Translational Perspective

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Dear Sir

Genetic alterations are considered to play an important role in both biological behavior and progression of human malignancies. However, cancer of gallbladder (CAGB) is an obscure phenomenon and is highly malignant with underprivileged diagnosis and poor survival (Chan et al., 2003). Earlier, we reported a significant higher prevalence of CAGB amongst victims of Bhopal gas tragedy exposed to methyl isocyanate (Mishra et al., 2009). This necessitated us to undertake a separate and detailed retrospective characterization of archived CAGB tissue samples for validating potential biomarkers for translational modalities. Tissues from 92 cases of CAGB (31 men and 61 women, age range 16–85 yrs, mean age 45.83 ± 1.50 yrs) with 70 adenocarcinoma (13 well differentiated, 48 moderately differentiated and 09 poorly differentiated), 10 adenosquamous carcinoma and 12 gallbladder adenoma were examined for Kirsten rat sarcoma viral oncogene homolog protein (K-ras), p-53, cyclin-E and Rad-50 expression through immuno-histofluorescence using spectral bio-imaging approach (Mishra et al., 2009). Microsatellite instability (MSI) was determined from PCR amplifications of six-microsatellite marker loci (D16S539, D13S317, D7S820, F13A01, FES/FPS, vWA) using an in-house standardized protocol. Cases were classified as having high-frequency MSI (MSI-H) (>/= 2 loci showing instability), low-frequency MSI (MSI-L) (only one locus showing instability), or as microsatellite stable.

Recent research has demonstrated that presence of K-ras mutation may influence diagnosis and clinical management of the patients with CAGB (Kamisawa et al., 2009). In support of our previous study, cases of adenocarcinomas occupied the major share of mutations and analysis of K-ras displayed alteration in its expression pattern among 55 of 70 adenocarcinoma (Figure 1a), with significant frequency being 78.6% (P<0.0001). Simultaneous analysis for p-53, Rad-50 and cyclin-E proteins showed analogous mutational frequency in 51 of 70 (73%), 28 of 70 (40%) and 23 of 70 (32.8%) respectively (P<0.0001).

Of all adenocarcinomas, the expression frequency of above four genes was higher in moderately differentiated adenocarcinoma in comparison to poorly and well differentiated ones. The positivity of mutations in adenosquamous cell carcinoma for above four genes were

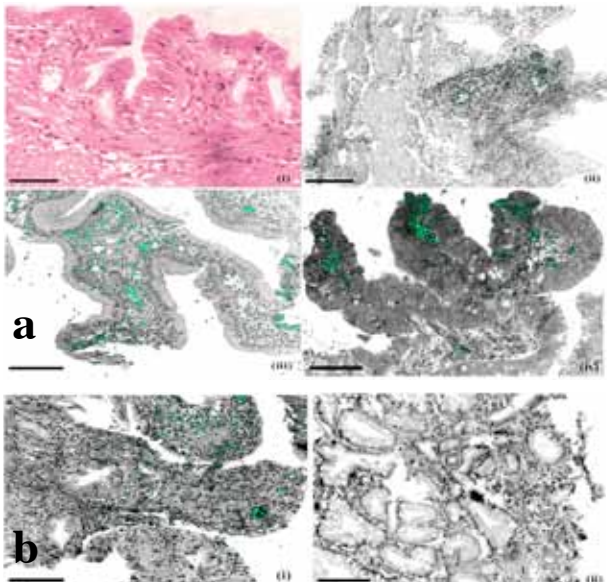


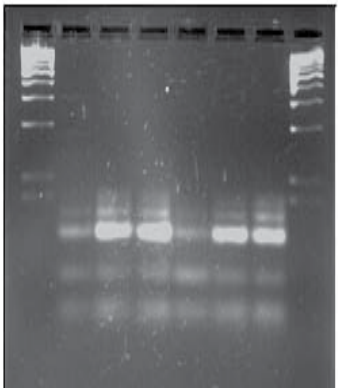
Figure 1. K-ras Protein Expression. a) Over expression in adenocarcinoma. Photomicrograph representing hematoxylin and eosin image of K-ras overexpression (i), immunohistofluorescence detection of k-ras protein overexpression in the nuclei of well differentiated adenocarcinoma (ii) moderately differentiated adenocarcinoma (iii), poorly differentiated adenocarcinoma (iv) of gallbladder. (Green spots: positive nuclear staining; DAPI counter-stained tissue: grey color) (K-ras; original magnification 200 X). (b) Immunohistofluorescence detection of k-ras protein overexpression in adenosquamous carcinoma (i), and adenoma with dysplasia (ii) of gallbladder. (Green spots: positive nuclear staining in background of DAPI counter-stained tissue: grey color) (K-ras; original magnification 200 X)

40%, 40%, 30%, and 1% respectively (P<0.0001) (Figure 1), suggesting the invasiveness of the disease. However, co-expression of K-ras, Rad-50 and Cyclin-E with p-53 were absent in adenomas with dysplasia implicating their independent role (Table 1). Microsatellite instability (MSI) is the molecular fingerprint of a deficient mismatch repair system. Analysis of microsatellites revealed instability amongst markers (D13S317, FES/FPS and F13A01) with frequency 10% (7/70) MSI-H in cases which belonged to moderate adenocarcinoma (Figure 2), however, we found no evidence of instability in adenomas suggestive that the replication errors may occur during the development of carcinomas but seldom

Table 1. Correlation of K-ras, p53, Rad50 and Cyclin E Overexpression in Gallbladder Carcinoma

	n	K-ras		p-53		Rad-50		Cyclin-E		P Value
		+ ve	%	+ ve	%	+ ve	%	+ ve	%	
Histological subtype	92									
Adenocarcinoma	70	55	78.6	51	73.0	28	40.0	23	32.8	P<0.0001
Adenosquamous carcinoma	10	4	40.0	4	40.0	03	30.0	1	1.0	P<0.0001
Adenomas with dysplasia	12	5	41.6	2	16.6	01	8.3	0	0.0	NS
Differentiation grade	70									
Well differentiated	13	7	54.0	8	61.5	04	30.7	03	23.0	
Moderately differentiated	48	37	77.0	39	81.0	23	47.9	22	45.8	
Poorly differentiated	09	4	44.4	3	33.3	02	22.2	04	44.4	

NS: Non significant



1 2 3 4 5 6 7 8

Figure 2. Microsatellite Instability. Microphotograph displaying the variant banding pattern of simple tandem repeat loci in CAGB subjects. Lanes 1 and 8: low base pair marker (100 bp); Lane 2: D16S539; Lane 3: D13S317; Lane 4: D7S820; Lane 5: F13A01; Lane 6: FES/FPS; lane 7: vWA.

in adenomas. These results imply that expression patterns of p-53, Rad-50, Cyclin-E and K-ras genes were altered along with mixed chimerism of STR loci. Results of our study establish Rad-50 and cyclin-E as potential biomarkers for early diagnosis of the disease hitherto unreported. Investigations are in progress to undertake similar studies on archived tumor tissues of varied origins and forms which might help in identifying and validating potential biomarkers for early prognosis of the carcinogenesis. These might also provide modalities to translate robust and reproducible strategies for defined clinical utility.

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Pradyumna K Mishra[1,2]*, Gorantla V Raghuram[1,2], Suresh K Jatawa[1], Arpit Bhargava[1,2], Subodh Varshney[1]

*[1]Bhopal Memorial Hospital & Research Centre, Bhopal, [2]Division of Translational Research, Tata Memorial Centre, ACTREC, Navi Mumbai, India *For correspondence: pkm_8bh@yahoo.co.uk*