

Supplementary Materials

Methylation Dynamics of *RASSF1A* and Its Impact on Cancer

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Table S1. *RASSF1A* methylation in liver neoplasms.

Cancer Type *	Rate of <i>RASSF1A</i> Methylation. Assay Location. Notes.	Detection Method	Association with Clinical Data	References
HCC	15 of 17 (88%) cancers. 2 of 7 (29%) cirrhotic tissues. Mean methylation level: 59% in 20 HCC, 26% in 9 cirrhotic, 16% in 12 non-cirrhotic tissues. 5 CpGs in first exon.	DNA pyrosequencing	YES	Araujo, O.C. et al., 2016 [1]
HCC	67 of 120 (85.5%) cancers. 48 of 120 (61.8%) normal cancer-adjacent liver. 9 of 35 (25.5%) hepatitis. 8 of 35 (23.6%) cirrhosis. 43 of 120 (36.3%) non liver tissues. Promoter, first exon.	Bisulfite DNA sequencing and quantitative MSP	n.d.	Jain, S. et al., 2015 [2]
HB	25 of 74 (34%) cancers. Promoter, first exon.	DNA pyrosequencing	YES	Honda, S. et al., 2013 [3]
HCC	82 of 103 (80%) cancers. Location unknown. <i>RASSF1A</i> methylation inversely correlated with mRNA and protein expression.	n.a.	NO	Feng, Y. et al., 2012 [4]
HCC	105 of 138 (76%) cancers. Mean methylation level: 53% in 138 HCC, 24% in 75 cirrhotic, 22% in 40 non-cirrhotic tissues, 10% in 28 healthy liver. 5 CpGs in first exon. Strong downregulation of <i>RASSF1A</i> mRNA expression compared to healthy liver.	DNA pyrosequencing	YES	Lambert, M.P. et al., 2011 [5]
HCC	31 of 35 (89%) cancers. Location unknown.	MSP	YES	Hu, L. et al., 2010 [6]
HCC	25 of 29 (86%) cancers. First exon. Loss of <i>RASSF1A</i> mRNA expression in 36% of HCC without correlation with methylation.	MSP	YES, better prognosis.	Saelee, P. et al., 2010 [7]
HB	6 of 20 (31%) cancers by MSP. 43 of 97 (44%) cancers by qMSP. Promoter, first exon.	MSP and quantitative MSP	NO by MSP. YES by qMSP.	Honda, S. et al., 2008 [8]
HB	15 of 39 (39%) cancers. Promoter, first exon.	MSP and bisulfite DNA sequencing	YES	Sugawara, W. et al., 2007 [9]
GC	5 of 22 (27%) cancers. Promoter, first exon. Reduction or loss of <i>RASSF1A</i> expression was observed in most methylated GC.	MSP	n.d.	Kee, S.K. et al., 2007 [10]

GC	4 of 50 (8%) cancers. Promoter, first exon.	MSP	n.d.	Riquelme, E. et al., 2007 [11]
HCC	26 of 26 (100%) tissue. Promoter.	MSP	n.d.	Di Gioia, S. et al., 2006 [12]
HCC	6 of 20 (30%) cancers. First exon.	MSP	n.d.	Oliveira, C. et al., 2005 [13]
HCC	37 of 40 (93%) cancers. First exon. 17 of 40 (43%) in the plasma of the same patients.	MSP	n.d.	Yeo, W. et al., 2005 [14]
GC	10 of 37 (27%) cancers. First exon.	MSP	NO	Tozawa, T. et al., 2004 [15]
GC	0 of 50 (0%) cancers. Promoter, first exon.	MSP	n.d.	Takahashi, T. et al., 2004 [16]
HCC	41 of 43 (95%) in cancer. 16 of 23 (70%) in matched normal liver tissue. 16 CpGs in the promoter.	Bisulfite DNA sequencing	n.d.	Zhong, S. et al., 2003 [17]
HCC	71 of 83 (85%) cancers. Promoter.	MSP	n.d.	Zhang, Y.J. et al., 2002 [18]
HCC	14 of 15 (93%) cancers. 2 of 2 (100%) fibrosis. 3 of 4 (75%) cirrhosis. First exon.	COBRA	n.d.	Schagdarsurengin, U. et al., 2003 [19]
CC	9 of 13 (69%) cancers. First exon. Reduced <i>RASSF1A</i> mRNA expression in 9 CC cases.	MSP	n.d.	Wong, N. et al., 2002 [20]

* HCC, hepatocellular carcinoma; GC, gallbladder carcinoma; HB, hepatoblastoma; CC, cholangiocarcinoma; MSP, methylation-specific PCR; COBRA, combined bisulfite restriction analysis; n.a., not available; n.d., not determined.

Table S2. *RASSF1A* methylation in esophageal squamous cell carcinoma.

Cancer Type *	Rate of <i>RASSF1A</i> Methylation. Assay Location. Notes.	Detection Method	Association with Clinical Data	References
ESSC	35 of 71 (46.5%) cancers. 1 of 35 (2.9%) in normal cancer-adjacent mucosa. Lower <i>RASSF1A</i> expression in cancer than in normal.	MSP	YES	Guo, Q. et al., 2016 [21]
ESCC	45 of 100 (45%) cancers. First exon.	MSP	n.d.	Du, Z. et al., 2015 [22]
ESCC	78 of 141 (55%) cancers. 6 of 141 (4%) normal adjacent tissues. First exon. Decreased mRNA and protein expression of <i>RASSF1A</i> was observed in ESCC and was associated with promoter and exon methylation status.	MSP	YES	Guo, W. et al. 2014 [23]
ESCC	76 of 143 (53%) cancers. 10 of 62 (16%) normal adjacent cancers. First exon. No association between <i>RASSF1A</i> methylation and protein expression.	MSP	YES	Zhou, S.L. et al., 2013 [24]
ESCC	79 of 124 (64%) cancers. First exon. <i>RASSF1A</i> hypermethylation was statistically associated with loss of mRNA expression.	Quantitative MSP	YES	Mao, W.M. et al., 2011 [25]
ESCC	7 of 50 (17%) cancers. Promoter and first exon	MSP	n.d.	Kim, Y.T. et al., 2009 [26]
ESCC	32 of 66 (49%) cancers. Site unknown.	MSP	n.d.	Cong, D.G. and Wang, S.F., 2007 [27]
ESCC	22 of 64 (34%) cancers. Promoter and first exon	MSP	NO	Wong, M.L. et al., 2006 [28]
ESCC	3 of 55 (24%) cancers. Promoter and first exon	MSP	n.d.	Yamaguchi, S. et al., 2005 [29]
ESCC	11 of 22 (51%) cancers. Promoter and first exon.	MSP	n.d.	Kuroki, T. et al., 2003 [30]
ESCC	25 of 48 (52%) cancers. Promoter and first exon.	MSP	n.d.	Kuroki, T. et al., 2003 [31]

* ESCC, esophageal squamous cell carcinoma; MSP, methylation-specific PCR; n.d., not determined.

Table S3. *RASSF1A* methylation in pancreatic neoplasms.

Cancer Type *	Rate of <i>RASSF1A</i> Methylation. Assay Location. Notes.	Detection Method	Association with Clinical Data	References
PDAC	3 of 14 (26%) xenografts cancers. Promoter and first exon. <i>RASSF1A</i> mRNA expression always detected independently from methylation status. Protein expression lower in cancer than normal in 50% of PDAC.	DNA pyrosequencing	n.d.	Amato, E. et al., 2016 [32]
PET	16 of 20 (80%) cancers by MSP. 11 of 20 (55%) cancers by DNA pyrosequencing. Promoter, first exon. <i>RASSF1A</i> mRNA level was lower in PET than normal and correlated inversely with average methylation in PET and normal.	MSP and DNA pyrosequencing	n.d.	Malpeli, G. et al., 2011 [33]
PET	54–80% in specific subtypes.	MSP	n.d.	Arnold, C.N. et al., 2007 [34]
PET	10 of 16 (63%) cancers. First exon.	MSP	n.d.	Liu, L. et al., 2005 [35]
PET	10 of 10 (100%) cancers. First exon.	MSP	n.d.	Pizzi, S. et al., 2005 [36]
PDAC	29 of 45 (64%) cancers. First exon. <i>RASSF1A</i> silenced in methylated cases and cell lines.	MSP	n.d.	Dammann, R. et al., 2003 [37]
PET	10 of 12 (83%) cancers. First exon.	MSP	n.d.	Dammann, R. et al., 2003 [37]
PET	36 of 48 (75%) cancers. First exon.	MSP	YES	House, M.G. et al., 2003 [38]

* PDAC, pancreatic ductal adenocarcinoma; PET, pancreatic endocrine tumor; MSP, methylation-specific PCR; n.d., not determined.

Table S4. *RASSF1A* methylation in colorectal cancer.

Cancer Type *	Rate of <i>RASSF1A</i> Methylation. Assay Location. Notes.	Detection Method	Association with Clinical Data	References
CRC	Higher methylation level in 100 CRC tissues compared to normal adjacent mucosa. Promoter.	DNA pyrosequencing	n.d.	Sugai, T. et al., 2017 [39]
CRC	18 of 51 (35%) cancers. 43 of 51 (85%) of paired liver metastases. Site unknown.	Quantitative MSP	n.d.	Schiroso, L. et al., 2016 [40]
CRC	42 of 65 (65%) cancers. 10 of 65 (15%) normal mucosa. Promoter.	MSP	YES	Li, M. et al., 2016 [41]
CRC	10 of 80 (12%) cancers. 1 of 80 (1%) adjacent normal mucosa. First exon.	Methylation-sensitive high resolution melting	YES	Coppede, F. et al., 2014 [42]
CRC	29 of 62 (47%) cancers. Promoter, first exon.	MSP	YES	Sinha, R. et al., 2013 [43]
CRC	16 of 111 (14%) cancers, average methylation 31%. Normal mucosa never methylated. Promoter, first exon.	DNA pyrosequencing	YES	Nilsson, T.K. et al., 2013 [44]
CRC	86 of 117 (74%) cancers. Site unknown.	n.a.	NO	Chen, S.P. et al., 2012 [45]
CRC	20 of 100 (20%) cancers. 9% in normal adjacent tumor cancers having verage methylation > 10%. First exon.	Quantitative PCR		Kang, H.J. et al., 2012 [46]
CRC	17 of 36 (47%) cancers. First exon.	MSP	NO	Abouzeid, H.E. et al., 2011 [47]
CRC	26 of 94 (28%) cancers. Methylation >15% in 22 CpGs. Normal mucosa: average methylation 18% (5-34%). No difference at 2 cm and 10 cm from cancer. Average methylation higher in distal than proximal normal mucosa. First exon.	DNA pyrosequencing and bisulfite DNA sequencing	NO	An, B. et al., 2010 [48]
CRC	8 of 47 (17%) cancers. 1 of 47 (2%) normal mucosa. First exon.	Quantitative PCR	YES	Gonzalo, V. et al., 2010 [49]
CRC	26 of 73 (36%) cancers. 14% in paired normal tissues. First exon.	MSP	NO	Miladi-Abdennadher, I. et al., 2010 [50]
CRC	10 of 12 (83%) cancers. 9 of 13 (69%) of normal colonic mucosa. Promoter.	MSP	n.d.	Minoo, P. et al., 2006 [51]
CRC	6 of 25 (24%) cancers. First exon. <i>RASSF1A</i> mRNA expression lower in methylated cases.	MSP	n.d.	Greenspan, E.J. et al., 2006 [52]
CRC	40 of 202 (20%) cancers. First exon.	MSP	n.d.	Miranda, E. et al., 2006 [53]
CRC	16 of 31 (52%) sporadic MSI cancers. First exon.	MSP	n.d.	Oliveira, C. et al., 2005 [13]
CRC	12 of 28 (43%) cancers. First exon.	MSP	n.d.	Dong, S.M. et al., 2005 [54]
CRC	39 of 48 (81%) cancers. 19 of 39 (49%) normal colonic mucosa. First exon.	MSP	n.d.	Sakamoto, N. et al., 2004 [55]
CRC	2 of 65 (3%) cancers. First exon.	MSP	n.d.	Xu, X.L. et al., 2004 [56]
CRC	45 of 220 (20%) cancers. First exon.	MSP	n.d.	van Engeland, M. et al., 2002 [57]

* CRC, colorectal carcinoma; MSP, methylation-specific PCR; n.a., not available; n.d., not determined.

Table S5. *RASSF1A* methylation in gastric cancers.

Cancer Type *	Rate of <i>RASSF1A</i> Methylation. Assay Location. Notes.	Detection Method	Association with Clinical Data	References
GC	14 of 21 (67%) cancers and 9 of 26 (35%) normal adjacent cancers (with metaplasia). First exon. <i>RASSF1A</i> expression was significantly reduced in methylated cases.	MSP	n.d.	Joo, M.K. et al., 2015 [58]
GC	13 of 102 (13%) cancers. First exon.	MSP	NO	Li, Y. et al., 2015 [59]
GCA	60 of 92 (65%) cancers. 6 of 30 (20%) normal adjacent cancer. First exon.	MSP	YES	Zhou, S.L. et al., 2013 [24]
GC	0 of 98 (0%) cancers. Average methylation 4.3% in 6 CpGs. First exon.	DNA pyrosequencing	NO	Balassiano, K. et al., 2011 [60]
GCA	54 of 92 (59%) cancers. Methylation in cancer higher than normal tissues. Promoter. Reduced <i>RASSF1A</i> mRNA and protein expression in cancer compared to normal tissues.	MSP	n.d.	Guo, W. et al., 2009 [61]
GC	0 of 16 (0%) cancers. No methylation in normal, metaplasia and displasia. First exon. No reduction of <i>RASSF1A</i> protein expression was observed in cancer compared to normal.	MSP	NO	Zou, X.P. et al., 2009 [62]
GC	36 of 54 (67%) cancers. <i>RASSF1A</i> methylation was higher in cancer than in corresponding normal tissues (66.7% vs. 14.8%). Promoter, first exon. <i>RASSF1A</i> mRNA and protein expression was reduced in cancer compared to normal tissues.	MSP	n.d.	Ye, M. et al., 2007 [63]
GC	11 of 25 (44%) cancers. First exon.	MSP	n.d.	Oliveira, C. et al., 2005 [13]
GC	6 of 80 (7.5%) cancers. Never found in adenomas, metaplasia, gastritis. First exon.	MSP	n.d.	Kang, G.H. et al., 2003 [64]
GC	39 of 90 (44%) cancers. 0 of 15 (0%) normal tissues. Promoter. Low level of <i>RASSF1A</i> mRNA in 46% and complete loss in 13% of 90 GC. No <i>RASSF1A</i> expression in cases and cell lines with LOH.	MSP, bisulfite DNA sequencing.	YES	Byun, D.S. et al., 2001 [65]

* GC, gastric carcinoma; GCA, gastric cardia adenocarcinoma; MSP, methylation-specific PCR; n.d., not determined.

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