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Genome-wide expression profile of sporadic gastric cancers with microsatellite instability

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ABSTRACT

Gastric cancers with mismatch repair (MMR) inactivation are characterised by microsatellite instability (MSI). In this study, the transcriptional profile of 38 gastric cancers with and without MSI was analysed. Unsupervised analysis showed that the immune and apoptotic gene networks efficiently discriminated these two cancer types. Hierarchical clustering analysis revealed numerous gene expression changes associated with the MSI phenotype. Amongst these, the p53-responsive genes maspin and 14-3-3 sigma were significantly more expressed in tumours with than without MSI. A tight immunosurveillance coupled with a functional p53 gene response is consistent with the better prognosis of MSI cancers.

Frequent silencing of MLH1 and downregulation of MMR target genes, such as MRE11 and MBD4, characterised MSI tumours. The downregulation of SMUG1 was also a typical feature of these tumours. The DNA repair gene expression profile of gastric cancer with MSI is of relevance for therapy response.

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1. Introduction

Gastric cancers (GC) with defective mismatch repair (MMR) comprise 10–25% of all GC. These tumours accumulate DNA replication errors at short-repeat sequences that are identified by the presence of microsatellite instability (MSI) (reviewed in [1] and references therein). MSI is mostly associated with hypermethylation of the MLH1 promoter CpG island leading to loss of MLH1 expression in the tumour and occasionally in the surrounding gastric mucosa.[2] MMR inactivation leads to accumulation of mutations in repeat tracts located within the coding and non-coding regions of tumour suppressor, anti-apoptotic and DNA repair genes as reported in both colon (reviewed in [3]) and gastric (reviewed in [1]) cancers with MSI. Defects in DNA repair pathways may impact on the tumour response to therapy (reviewed in [4]).

Recent data on gene expression profiling of endometrial[5] and colon[6] cancers with and without MSI support the hypothesis that these cancer subsets arise through distinct pathways of aberrant signalling. These pathways are likely cancer

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specific as indicated by the lack of common signature between MSI endometrial and colon cancers with hypermethylation of MLH1 as the only consistently observed feature.

The main objective of this study was to determine whether the MSI phenotype in GC could be distinguished from the microsatellite stable (MSS) phenotype using microarrays. Here, we show that MSI GC can indeed be discriminated from MSS tumours by their specific gene expression signature that mainly involves the immune response and the p53 gene network. Moreover, the DNA repair gene expression profiling showed that besides MLH1 silencing that characterised the majority of MSI GC, a set of DNA repair genes were differentially expressed in these tumours too. This information should be used to design therapies tailored to this GC subgroup.

2. Materials and methods

2.1. Patients and clinical specimens

All tissues were collected from a series of GC cases identified in an area around Florence (Italy) characterised by high GC risk. Following full institutional review board approval, patients were recruited in the period 2000–2005 from the main hospitals of this area. All patients were residing in the Florence district, and they signed an informed consent before providing a blood sample and a gastric tissue during surgery. Immediately after resection, tissues from the invasive region of cancer and normal adjacent mucosa were flash-frozen in liquid nitrogen. Tissues were cryopreserved until use. Gastric tumours were confirmed histologically, and were defined according to the Laurén classification for histological type and to the American Joint Committee on Cancer (AJCC) classification for depth of invasion into the gastric wall (pT) and involvement of regional lymphnodes (pN).

2.2. MSI status analysis

DNA was extracted from 131 fresh-frozen GC samples using standard procedures. The MSI status was analysed at the two highly sensitive mononucleotide markers, *BAT26* and *BAT25*, by a PCR-based assay using primer sets that were previously reported.[7] Tumours were classified as MSI when showing contractions in *BAT26* and *BAT25* and as MSI-negative when no instability was observed at the loci tested. Results were always confirmed using two independent DNA extracts. By this procedure, 19 GC with MSI were identified and matched with 19 MSS tumours by age, sex and grade (Table 1).

2.3. RNA isolation

The tissues were homogenised, and a maximum of 250 mg of the sample was subjected to RNA isolation using the RNeasy Midi Kit following the manufacturers' instructions (Qiagen, Valencia, CA, USA). Following the isolation of RNA, the integrity of each RNA sample was verified by Bioanalyser (Agilent Technologies) using RNA nanochips according to manufacturer's instructions. Only those RNAs that passed this quality control were subjected to array analysis.

2.4. Gene expression array

Biotinylated cRNA targets were synthesised from each sample and hybridised to Affymetrix oligonucleotide chips (GeneChip HG-U133A/B) that contain 45,000 probe sets (39,000 unique transcripts and 33,000 well-substantiated human genes) according to manufacturers' instruction (Affymetrix Inc., Santa Clara, CA). DAT files were analysed by MAS 5.0 to generate background-normalised image data (CEL files). Probe set intensities were obtained by means of the robust multiarray analysis method.[8] The full data set was normalised according to the invariant set method.[9] The expression data are available at GEO (Gene Expression Omnibus) public data bank http://www.ncbi.nlm.nih.gov/geo/.

Genes differentially expressed between MSI and MSS tumours were identified as follows: (i) gene probe sets were pre-selected for being flagged as 'PRESENT' in at least 10 tumour samples (21,324 probe sets); (ii) the probe set signal values on the pre-selected probe sets were used to run a *t*-test between the two types of tumour samples; (iii) the false discovery rate (FDR) method was applied to the *t*-test calculated *p*-values to correct for multiple testing, according to [10] and (iv) a false discovery rate <0.05 and a *t*-test *p*-value < 0.01 were chosen as threshold criteria to identify the genes differentially expressed in tumours with and without MSI (3866 probe sets). Microarray findings were confirmed by real-time reverse-transcription PCR (RT-PCR) using a pool of mRNA from normal gastric tissues as calibrator sample. Analysis of mRNA expression of some selected DNA repair genes (APEX1, MLH1, BRCA1, BRCA2, ERCC1, FEN1, LIG1, LIG3, LIG4, MBD4, MPG, MRE11A, MSH2, MSH3, MSH6, OGG1, PMS2, POLB, RAD51, SMUG1, UNG, XPC and XRCC1) was performed using the pre-designed low density array (Applied Biosystems) following the manufacturers' instructions. Expression values were computed using the comparative CT method (ΔΔCT) with GAPDH or 18S gene expression value serving as normaliser. Non-parametric tests (Mann–Whitney) were performed to test differences on distribution of expression values between MSI and MSS.

2.5. Statistical methods

Affymetrix gene expression data were submitted to PCA by using the genes as rows (statistical units) and the samples as columns (variables). The difference between groups was assessed by means of Student's *t*-test on component loadings. To generate a biological interpretation of the set of gene probes occupying the high and low extremes of the principal component, the probes having a low probability ($p < 0.001$) of belonging to a Gaussian fit to the distribution of scores along the principal component axis were selected and tested for gene ontology (GO) statistical enrichment.[11] The Fisher exact test *p*-value for each GO term was calculated.

Hierarchical clustering analysis was performed using MatLab 7.0. The algorithm was applied in an unsupervised manner to cluster (i) all samples and (ii) tumour samples only. In both cases, signal z-scores were calculated for each probe in each sample. Probe sets having z-score > 1.5 and a detection *p*-value < 0.01 in more than 10 samples were pre-selected and used for clustering using the Euclidean distance as a sim-

Table 1 – Clinical, pathological and molecular characteristics of gastric cancer cases.

ID	MSI status	Age	Sex	Lauren	TNM
17	MSS	72	F	I	T2N2Mx
22	MSS	79	M	I	T3N1Mx
39	MSS	74	F	I	T3N2M1
43	MSI	77	F	I	T2N1Mx
46	MSS	73	M	I	T3N1Mx
53	MSS	74	F	M	T3N3Mx
58	MSI	61	F	I	T3N1Mx
60	MSI	76	M	D	T3N1Mx
61	MSI	63	F	I	T3N1Mx
63	MSS	81	M	I	T2N1Mx
91	MSS	74	F	M	T2N1Mx
96	MSS	77	M	I	T3N1Mx
105	MSI	81	F	I	T3N0Mx
114	MSI	62	F	I	T2N3Mx
115	MSI	65	M	I	T2N1Mx
117	MSI	89	M	I	T3N1Mx
118	MSI	81	F	D	T3N1Mx
123	MSI	76	M	I	T2N1Mx
124	MSI	65	F	D	T3N3Mx
127	MSS	67	F	I	T3N1Mx
129	MSS	64	F	D	T1N0Mx
130	MSS	72	F	D	T3N2Mx
146	MSI	64	F	I	T3N2Mx
148	MSI	73	M	I	T3N1Mx
149	MSS	82	M	I	T2N1Mx
154	MSI	78	F	I	T2N0Mx
158	MSS	84	M	I	T3N0Mx
159	MSS	79	F	D	T3N0Mx
174	MSS	62	M	M	T3N3Mx
177	MSI	82	F	U	T3N1Mx
183	MSI	84	F	I	T3N0Mx
186	MSI	75	M	I	T3N0Mx
189	MSS	70	F	I	T2N1Mx
192	MSI	76	F	I	T2N0Mx
195	MSS	73	M	I	T3N1Mx
196	MSS	64	F	M	T3N1Mx
202	MSI	68	M	U	T3N0Mx
203	MSS	81	F	I	T3N1Mx

ID, case number; MSS, microsatellite stable; MSI, microsatellite unstable; SEX: M, male; F, female; LAUREN: Lauren classification (I, intestinal; D, diffuse; M, mixed; U, unclassified); TNM, TNM classification of malignant tumours (see Section 2).

ilarity measure between the samples. The unweighted average link was used to calculate the distance from a cluster to all remaining unclustered points.

3. Results

3.1. *Gene expression analysis discriminates gastric cancer specimens with and without MSI*

We first analysed 131 primary GC of similar grades, stages and histologies for the MSI phenotype by using two highly sensitive mononucleotide markers, *BAT26* and *BAT25*. Overall, 20/131 (15.3%) GC showed contractions in both *BAT26* and *BAT25*, and were thus classified as MSI. 19 MSI tumour samples were selected and matched by age, sex and grade with 19 MSI-negative (MSS) cancers characterised by lack of instability at both the loci tested. Demographic, clinical and histopathological features of these tumours are shown in Table 1. The age of patients ranges between 61 and 89 years, and the GC (all adenocarcinomas) are mostly intestinal-type and of stage III.

Gene expression array data generated from our set of 31-matched normal/tumour tissues and seven-unmatched tumour tissues were used to verify the ability of our procedure to segregate normal mucosa from cancer specimens. Unsupervised PCA and unsupervised clustering analysis effectively segregated the normal mucosa and the cancer specimens (Fig. 1, Supplementary Information), thus validating our procedure. We then addressed the question of whether the gene expression profile of gastric MSS specimens is discernable from that of their counterparts with MSI. On the entire data set (normal and tumour samples), PCA was able to discriminate MSI and MSS specimens but, interestingly, in the tumour subgroup only (data not shown). This is in agreement with the fact that microsatellite instability and the associated gene expression changes are a unique trait of tumour tissues. To further analyse this discrimination, PCA was conducted on the gene expression data of MSI and MSS tumour specimens (without correction for

expression of the corresponding normal mucosa). Globally a six-component solution (PC_t1–PC_t6) accounted for 95% of the total variability with a clear separation from the noise floor. PC_t2 explained 1.2% of gene expression variability, and contained all the information necessary for the discrimination between samples with and without MSI (PC_t2, MSS tumours versus MSI tumours, $p < 0.0001$, *t*-test). The plane spanned by PC_t2 and PC_t6 loadings allows the visualisation of the discrimination between MSS and MSI tumours (Fig. 2A). The set of gene probes occupying the high and low extremes of PC2 (Table 2, Supplementary Information) were subjected to GO statistical enrichment.[11] GOs that were enriched at a significance $p < 0.001$ are presented in Table 3. The immune response pathway including the interferon inducible transmembrane proteins (IFITM) family, the major histocompatibility complex (MHC) class I and II antigens, the human leukocyte antigen (HLA) class I antigen subunits and the antigen-binding immunoglobulin superfamily emerged as the most representative descriptors ($p = 2.1^{-16}$) of the MSI specimens. Other discriminating pathways were regulation of stress response, apoptosis, cell cycle and angiogenesis. Unsupervised hierarchical clustering also effectively segregated the cancer specimens with and without MSI (Fig. 2B) (p-value $< 10^{-3}$).

In order to identify potential signature genes of the MSI phenotype, the genes whose expression was significantly different between the two tumour types were identified (see Section 2), and compared with the genes occupying the high and low extremes of PC2. There was a significant overlapping between the two probe lists (Fischer exact test $p < 10^{-9}$), and the intersection identified a set of genes (Table 4) that mainly belonged to apoptosis and immune and stress responses. Amongst these genes, we focused our attention on two p53-induced genes: maspin (or serpin B5) and 14-3-3 sigma (or stratifin). In a subset of 22 specimens, these two genes were confirmed by RT-PCR analysis to differentiate tumours with and without MSI (Fig. 3) with significantly higher expression in MSI as compared to MSS tumours (3.1-fold versus 0.40-fold for maspin and 2.0-fold versus 0.45-fold for 14-3-3 sigma).

3.2. *MSI/MSS discrimination by DNA repair gene expression profile*

It is well known that the MSI phenotype associates with DNA repair defects. Low density arrays were used to monitor the expression of 23 genes belonging to different DNA repair pathways by RT-PCR. The MSI status exerted indeed an influence on the DNA repair gene expression profile of our sporadic GC. The loss of MLH1 was confirmed to be a significant event in the development of our MSI gastric tumours that were mostly characterised by silencing of this gene (Fig. 4). Interestingly, the levels of expression of MLH1 were strongly associated with the expression of known targets of the mutator phenotype such as MBD4 and MRE11 (Spearman rank correlation coefficient = 0.71 and 0.81, $p < 0.0001$, respectively), which significantly discriminated GC on the basis of their MSI phenotype (Fig. 4). We also report for the first time that the SMUG1 DNA glycosylase is significantly differentially expressed in MSI tumours versus MSS tumours (Fig. 4). This DNA glycosylase is involved in the removal of 5-fluorouracil (5-FU) that is widely used in the treatment of a range of cancers, including GC. XRCC1 expression levels were significantly correlated with those of MLH1 (Spearman rank correlation coefficient = 0.61, $p = 0.0001$) and, although not significantly, also with MSI status (Fig. 4). The other DNA repair genes (see Section 2) were not differentially expressed in the two tumour subgroups.

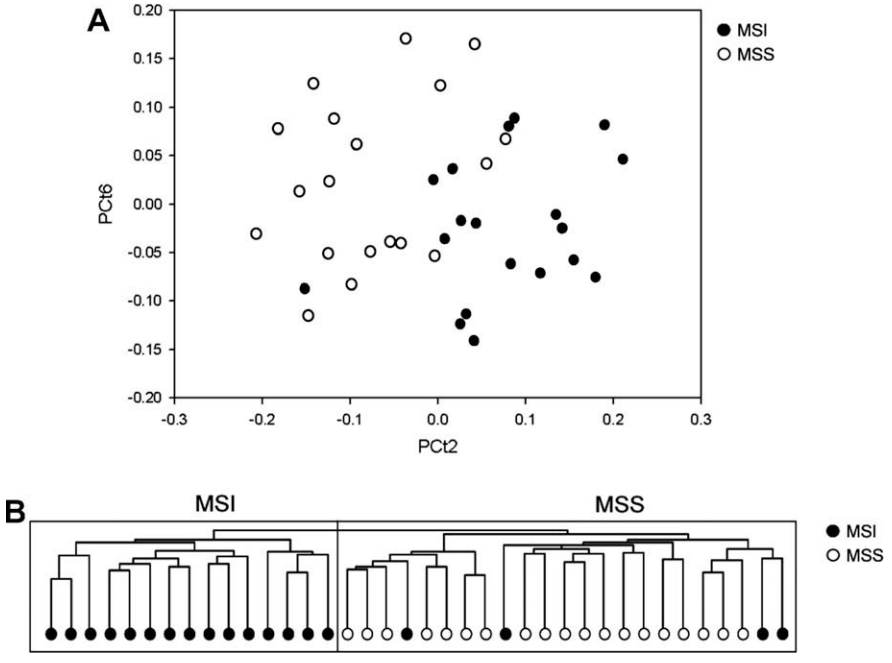

Fig. 2 – Analysis of gene expression profile of gastric tumours with and without MSI (A) Unsupervised PCA. (B) Hierarchical clustering trees of unsupervised analyses. MSI tumours (black circle) and MSS tumours (white circle).

Table 3 – Gene ontology (GO) pathways that differentiate MSI from MSS gastric tumours.

GO pathway	*p*-Value	PC2	Gene ID	Gene title
Defense response immune response antigen-binding response to endogenous stimulus/immune cell activation/response to virus/ chemotaxis/mitochondrial transport of proapoptotic proteins Bad and Bax	2.1E–16	21.408	5284	Polymeric immunoglobulin receptor
		16.837	8519	Interferon-induced transmembrane protein 1 (9–27)
		11.89	3576	Interleukin 8
		8.956	10581	Interferon-induced transmembrane protein 2 (1–8D)
		8.547	4282	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
		8.457	10410	Interferon-induced transmembrane protein 3 (1–8U)
		7.723	3429	Interferon, alpha-inducible protein 27
		6.379	2919	chemokine (C–X–C motif) ligand 1 (melanoma growth stimulating activity, alpha)
		–7.907	3135	HLA-G histocompatibility antigen, class I, G
		–8.122	7852	Chemokine (C–X–C motif) receptor 4
		–9.087	3115	Major histocompatibility complex, class II, DP beta 1
		–9.711	3495	Immunoglobulin heavy constant delta
		–10.082	972	CD74 molecule, major histocompatibility complex, class II invariant chain
		–10.116	3113	Major histocompatibility complex, class II, DP alpha 1
		–10.246	3106	Major histocompatibility complex, class I, B
		–10.678	5996	Regulator of G-protein signalling 1
		–12.183	3123	Major histocompatibility complex, class II, DR beta 1
		–14.118	3507	Immunoglobulin heavy constant mu
		–15.179	3122	Major histocompatibility complex, class II, DR alpha
		–19.698	3514	Immunoglobulin kappa constant
		–21.892	91353	Similar to omega protein
		–25.787	3512	Immunoglobulin heavy constant mu
Response to stress/response to hypoxia/response to oxidative stress/oxygen and reactive oxygen species metabolism	7.4E–12	19.593	2810	Stratifin
		12.475	7295	Thioredoxin
		12.177	302	Annexin A2
		11.99	6647	Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))
		8.777	3329	Heat shock 60 kDa protein 1 (chaperonin)
		7.889	3336	Heat shock 10 kDa protein 1 (chaperonin 10)
		7.082	7001	Peroxiredoxin 2
		6.486	7033	Trefoil factor 3 (intestinal)
		–7.82	2512	Ferritin, light polypeptide
		–12.247	7032	Trefoil factor 2 (spasmolytic protein 1)
		–15.778	6414	Selenoprotein P, plasma, 1
		–29.629	2165	Coagulation factor XIII, B polypeptide
Apoptosis/cell death	4.13E–06	15.78	3875	Keratin 18
		12.813	4680	Carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen) /// carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)
		10.458	5268	Serpin peptidase inhibitor, clade B (ovalbumin), member 5
		9.576	2597	Glyceraldehyde-3-phosphate dehydrogenase
		7.938	64065	PERP, TP53 apoptosis effector
		–7.676	1915	Eukaryotic translation elongation factor 1 alpha 1
		–9.057	10562	Olfactomedin 4
		–17.218	4069	Lysozyme (renal amyloidosis)
		11.337	8870	Immediate early response 3
		10.243	9168	Thymosin, beta 10
		8.183	9052	G-protein-coupled receptor, family C, group 5, member A
		6.844	801	Calmodulin 1 (phosphorylase kinase, delta)
		–14.883	7431	Vimentin
Cell proliferation/positive regulation of cell proliferation/ negative regulation of cell proliferation/cell cycle 2. E-8/ regulation of progression through cell cycle/cell cycle arrest/mitotic cell cycle	1.04E–8	11.12	6282	S100 calcium-binding protein A11 (calgizzarin)
		10.97	5757	Prothymosin, alpha (gene sequence 28)
		10.243	9168	Thymosin, beta 10
		9.337	6418	SET translocation (myeloid leukemia-associated)
		8.434	521	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit E
		7.219	7037	Transferrin receptor (p90, CD71) /// Transferrin receptor (p90, CD71)
		6.445	5967	Regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein)
		–7.692	10457	Glycoprotein (transmembrane) nmb

(*continued on next page*)

Table 3 – (*continued*)

GO pathway	*p*-Value	PC2	Gene ID	Gene title
		–8.029	4082	Myristoylated alanine-rich protein kinase C substrate
		–8.484	10628	Thioredoxin interacting protein
		–9.191	5500	Protein phosphatase 1, catalytic subunit, beta isoform
		–9.291	2	Alpha-2-macroglobulin
		–9.71	1634	Decorin
		–10.692	3490	Insulin-like growth factor binding protein 7
Cell adhesion	1.2E–04	10.004	1829	Desmoglein 2
		8.713	3934	Lipocalin 2 (oncogene 24p3)
		6.803	1499	Catenin (cadherin-associated protein), beta 1, 88 kDa
		6.635	1048	Carcinoembryonic antigen-related cell adhesion molecule 5
Regulation of angiogenesis	1.1E–05	–8.594	397	Rho GDP dissociation inhibitor (GDI) beta

4. Discussion

MSI characterises approximately 15% of sporadic colorectal and gastric cancers. This study is the first report on transcriptional profile of MMR defective GC conducted on numerous sets of MSI tumours matched with MSS tumours (by age, sex and grade). The pathways that better discriminated the MSI status were the immune and apoptotic responses. Besides genes involved in the proinflammatory response, several components of the antigen presentation and binding machinery were differentially expressed in MSI tumours versus MSS tumours. The latter included the MHC class I and II and HLA class I antigen molecules that are pivotal in the orchestration of an immune response. One decade ago mutations of β2-microglobulin leading to total loss of HLA I had been clearly associated with MSI in colorectal cancer.[12] More recently, defects in HLA class I antigen machinery in colorectal[13] and gastric[14] cancers with MSI have been reported. We specifically identified in MSI tumours downregulation of the non-classical HLA-G that is involved in the escape of tumour

Table 4 – List of genes differentially expressed in gastric cancers with and without MSI.

Gene ID	Gene name	Fold change MSI/MSS	Description
5268	SERPINB5	3.8	Serpin peptidase inhibitor that regulates cell migration in angiogenesis, and apoptosis
2810	SFN	3.2	Stratifin, regulates cell cycle, inhibits apoptosis
2919	CXCL1	3.1	Chemokine (C–X–C motif) ligand 1 involved in immune cell chemotaxis and inflammatory response
7033	TFF3	3.0	Intestinal trefoil factor 3 that contributes to mucosal defense
6275	S100A4	2.7	S100 calcium-binding protein A4 that regulates angiogenesis
304	ANXA2P2	2.3	Annexin A2 pseudogene 2
3576	IL8	2.2	Interleukin 8, a cytokine involved in immune response
200916	RPL22L1	2.2	Ribosomal protein L22 (human RPL22
8870	IER3	2.2	Immediate early response 3 of the apoptotic pathway
84419	C15orf48	2.1	Nuclear protein that may act as a putative tumour suppressor
7262	PHLDA2	2.0	Pleckstrin homology-like domain family A member 2, involved in blastocyst development
521	ATP5I	2.0	ATP synthase H+ transporting mitochondrial
10562	OLFM4	–2.0	Olfactomedin 4, an anti-apoptotic protein
28902	IGKV1D-13	–2.0	Immunoglobulin kappa variable 1D-13
3512	IGJ	–2.1	Immunoglobulin J chain
28904	IGKV1D-8	–2.3	Immunoglobulin kappa variable 1D-8
3514	IGKC	–2.3	Immunoglobulin kappa constant
4069	LYZ	–2.4	Lysozyme (renal amyloidosis
28823	IGLV1–44	–2.5	Immunoglobulin lambda variable 1–44
28815	IGLV2–14	–2.5	Immunoglobulin lambda variable 2–14
28923	IGKV2–24	–2.7	Immunoglobulin kappa variable 2–24
3135	HLA-G	–2.7	Class I histocompatibility antigen G
91353	CTA-246H3.1	–3.0	Protein containing an immunoglobulin C1-set domain
28786	IGLV4–3	–3.1	Immunoglobulin lambda variable 4–3
3493	IGHA1	–4.8	Immunoglobulin heavy constant alpha 1
3500	IGHG1	–4.8	Immunoglobulin heavy constant gamma 1 (G1m marker)
28396	IGHV4–31	–6.0	Immunoglobulin heavy variable 4–31
28461	IGHV1–69	–7.7	Immunoglobulin heavy variable 1–69

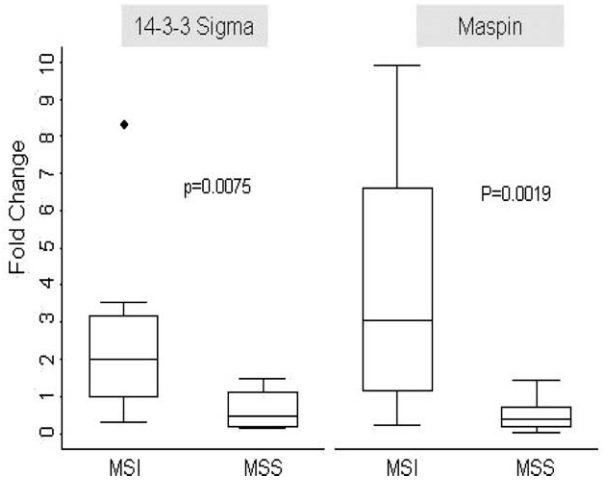

Fig. 3 – Box-whisker plot of expression levels of maspin and 14-3-3 sigma by MSI status. Mann–Whitney two-sample ranksum test was performed, and *p*-values are reported.

cells from immunosurveillance. Different profiles of immunoglobulin expression were also detected as marker of MSI status, likely indicating a differential vascular invasion in the two cancer types.

All together our findings provide a clear evidence that, as in the case of MSI colorectal tumours,[6] there is a tight immunologic surveillance of GC with MSI as documented by the selection of cells with defects of the immunosurveillance machinery.

Higher apoptosis/proliferation ratios and reduced cell proliferation are phenotypic features of MSI colon cancers.[15] Genes involved in the apoptosis and cell proliferation pathways are also present amongst the 'extreme' genes that discriminate GC with and without MSI. Some of the apoptosis-related genes that discriminate the MSI status belong to the DNA damage-induced p53 gene response network, such as Perp, calmodulin, maspin and 14-3-3 sigma. Two of these genes maspin and 14-3-3 sigma were also confirmed by RT-PCR to differentiate gastric tumours with and without MSI. Since both these genes are p53-responsive, their overexpression in MSI tumours suggests that p53 is fully functional in these tumours. No p53 mutations were indeed reported in colon[16] as well as in gastric[17] cancers with high MSI. Frequent hypermethylation of 14-3-3 sigma was detected in poorly differentiated GC, and the association with the chromosome instability (CIN) phenotype was suggested.[18] The downregulation of this gene is very frequent amongst our MSS samples, whereas this event is infrequent amongst tumours with MSI. Silencing of the maspin gene has been observed in many cancers due to promoter methylation.[19] As in the case of 14-3-3 sigma, this gene is often down-regulated amongst our MSS tumours. Its overexpression in MSI GC matches that previously reported in colon cancers with MSI.[6] On the basis of our findings, we can conclude that the role played by MMR defects in colorectal and gastric cancer aetiologies is very similar as suggested by the previous reports.[20] Recent evidence has been provided that high level of MSI in GC is associated with a higher survival at 15 years and with a loss of MLH1.[21] Whether the immune and apoptotic pathways are involved in the overall better prognosis of GC should be evaluated with *ad hoc* studies.

As expected, *MLH1* gene was significantly down-regulated in the MSI cancer set versus the MSS cancer set. The lack of MLH1 in the list of the discriminating genes generated by PCA is likely explained by (i) the different sizes of the gene networks involving DNA repair members as compared with those implicated in the immune response and (ii) the filter for correlated information that is imposed by PCA. However, the fact that the expression of MLH1 is the discriminating factor for the allocation of samples in different classes (as identified by PCA) suggests that it is the expression of this gene that drives the transcriptome of MSI cancers (i.e. changes in the expression of immune and apoptotic gene pathways).

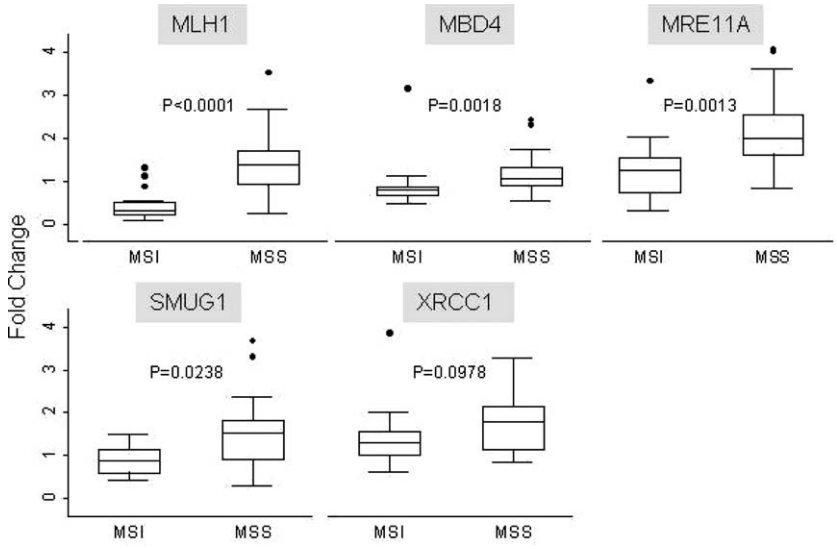

Fig 4 – Box-whisker plot of expression levels of selected DNA repair pathway genes by MSI status. Mann–Whitney two-sample ranksum test was performed, and *p*-values are reported.

The analysis of the expression of genes belonging to DNA repair as a function of the MSI status uncovered other interesting features of GC. MRE11, MBD4 and SMUG1 expression levels significantly discriminated GC on the basis of their MSI status. A correlation between XRCC1 and MLH1 expressions was also detected. XRCC1 had been previously reported to correlate with MSI status in colorectal cancers.[22] MRE11 belongs to the DNA double strand break (DSB) signalling and repair by interacting with Rad 50 and NBS1 within the MRN complex. DSB may be generated by endogenous damage, thus representing an important threat to gastric cells. Mutations in non-coding repeats that affect protein expression have been described in GC with MSI (reviewed in [1]). Similarly, mutations affecting the methyl-CpG binding thymine glycosylase MBD4 have been described at high frequency in sporadic colon cancer with MSI.[23] The occurrence of frameshift mutations that lead to truncation of MBD4 is compatible with events caused by MMR. The expression levels of both MRE11 and MBD4 are significantly associated with the levels of expression of MLH1, thus strengthening a possible causal link between MMR inactivation and inactivation of gene targets. We show that these mutations are associated with substantial loss of the transcripts. The finding of a significant discrimination between MSI and MSS tumours on the basis of the expression levels of SMUG1 deserves a comment. 5-FU is one of the most important chemotherapeutics for sporadic colon and gastric cancers. There is an important evidence that supports a relative lack of efficacy of 5-FU in patients with MMR defective tumours.[24] The recent discovery that besides MMR various DNA glycosylases particularly SMUG1 uracil DNA glycosylase play an important role in the repair of 5-FU incorporated into DNA[25] opens the questions of the mechanistic basis of this resistance. The inactivation of MMR and BER (via inactivation of MLH1 and SMUG1) as seen in our MSI tumours is compatible with the current model, where 5-FU cytotoxicity is due to futile cycles of MMR and BER that together with nucleotide pool depletion would lead to cytotoxic gaps or breaks in DNA.[26]

In summary, we have shown that GC with MLH1 silencing presents a characteristic transcriptional profile, where the immune response and an efficient p53 gene response are the likely mechanistic bases for long-term survival. The translation of the DNA repair gene expression profile of these tumours to clinical practice remains a challenging objective that should be pursued.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.10.032.

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