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Genome-Wide Analysis of Pancreatic Cancer Using Microarray-Based Techniques

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Key Words
Pancreatic ductal adenocarcinoma · Array-based comparative genomic hybridisation · High-level amplification · Homozygous deletion · Non-random genetic alterations

Abstract
Background/Aims: Microarray-based comparative genomic hybridisation (CGH) has allowed high-resolution analysis of DNA copy number alterations across the entire cancer genome. Recent advances in bioinformatics tools enable us to perform a robust and highly sensitive analysis of array CGH data and facilitate the discovery of novel cancer-related genes. ***Methods:*** We analysed a total of 29 pancreatic ductal adenocarcinoma (PDAC) samples (6 cell lines and 23 microdissected tissue specimens) using 1-Mb-spaced CGH arrays. The transcript levels of all genes within the identified regions of genetic alterations were then screened using our Pancreatic Expression Database. ***Results:*** In addition to 238 high-level amplifications and 35 homozygous deletions, we identified 315 minimal common regions of 'non-random' genetic alterations (115 gains and 200 losses) which were consistently observed across our tumour samples. The small size of these aberrations (median size of 880 kb) contributed to the reduced number of candidate genes included (on average 12 Ensembl-annotated genes). The database has further specified the genes whose expression levels are consistent with their copy number status. Such genes were *UQCRB*, *SQLE*, *DDEF1*, *SLA*, *ERICH1* and *DLC1*, indicating that these may be potential target candidates within regions of aberrations. ***Conclusion:*** This study has revealed multiple novel regions that may indicate the locations of oncogenes or tumour suppressor genes in PDAC. Using the database, we provide a list of novel target genes whose altered DNA copy numbers could lead to significant changes in transcript levels in PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in Western countries [1]. The extremely high mortality rate of patients with PDAC is clinically considered to be due to late diagnosis and lack of effective therapeutic strategies, but biologically its aggressiveness could be explained by the high level of genomic instability in PDAC cells [2–6]. In general, genomic aberrations are selected for the induction of tumour growth advantage and accumulated according to tumour progression [7–10]. The availability of microarray-based comparative genomic hybridisation (CGH) technology has allowed genome-wide, high-throughput screening for DNA copy number alterations in cancer cells [11–13]. Among those changes identified,

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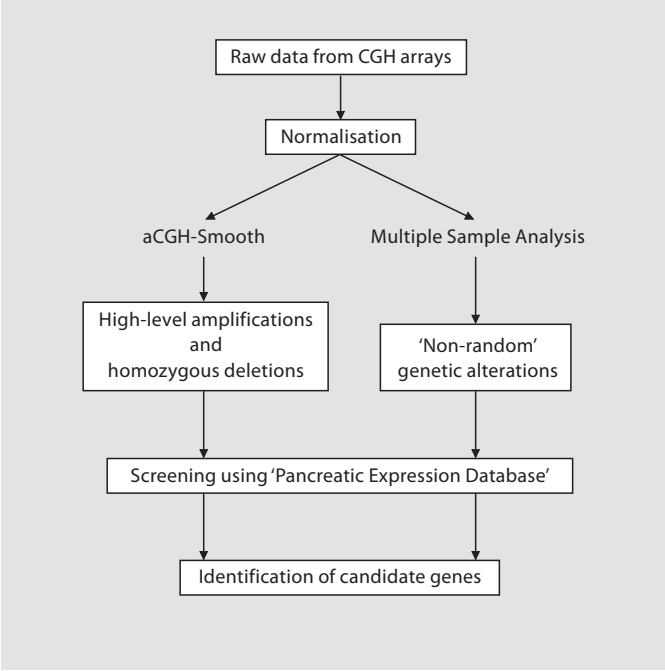

Fig. 1. A schematic overview of our microarray-based screening strategy to identify novel candidates involved in pancreatic carcinogenesis.

it is well known that high-magnitude alterations such as high-level amplifications (HAs) or homozygous deletions (HDs) could transform tumour cells to a more aggressive phenotype through amplification-induced overexpression of oncogenes or inactivation of tumour suppressor genes, respectively [14–20]. Furthermore, recent studies have demonstrated that 'non-random' genetic changes, which are consistently observed across a specific tumour type, should also be highlighted: these aberrations, even if low-level, could lead to deregulation in the expression levels of the genes included, and therefore may indicate the locations of novel oncogenes or tumour suppressor genes that play a critical role in carcinogenesis [21–24].

Here, we introduce our microarray-based approaches to screen for novel candidate genes involved in the pathogenesis of PDAC (fig. 1). We have analysed a total of 29 PDAC samples (6 cell lines and 23 microdissected tissue samples) using 1-Mb-spaced CGH arrays to identify two different types of genomic aberrations: (1) high-level copy number changes (i.e. HAs and HDs) and (2) non-random genetic alterations. Then, using the database that allows the rapid survey of transcript levels of the genes in PDAC, we provide a list of novel target genes affected by genomic aberrations.

Materials and Methods

Tumour and Reference Samples

Six cell lines (Panc1, PaTu1, MiaPaCa2, SUIT2, Cfpac1 and RWP1) were acquired from Cancer Research UK Cell Services. A series of 23 fresh-frozen PDAC tissue specimens which have previously been used was also included in this study (online suppl. table 1; for suppl. material see www.karger.com/doi/000178871) [5]. In tissue specimens, microdissection was manually performed to collect more than 90% purity of tumour cells. DNA was extracted as described previously [3, 5]. Reference DNA was obtained from lymphocytes of both healthy male and female Japanese volunteers. All the clinical samples were obtained with ethical approval of the host institutions.

CGH Arrays and Data Analysis

The whole-genome CGH arrays were produced at the Wellcome Trust Sanger Institute and consist of ~3,125 BAC/PAC clones that cover the entire human genome at approximately 1 Mb resolution [25]. Array CGH was performed as described previously [5]. Briefly, tumour and reference DNAs (300 ng) were labelled with Cy5-dCTP and Cy3-dCTP, respectively. Hybridisation was carried out at 37°C for 36 h in a hybridisation chamber (GeneMachines, San Carlos, Calif., USA). After washing the slides, fluorescence intensities were measured on an Axon 4000B scanner (Axon Instruments Inc., Burlingame, Calif., USA), and the raw images were analysed using the GenePix Pro 4.0 software (Axon). Grid placement, spot quality, data normalisation and fluorescence quantification of the array images were done using the UCSF 'SPOT' software [26].

Identification of Genetic Alterations in Each Case

Firstly, 'aCGH-Smooth' was used to detect DNA copy number alterations in each tumour sample [27]. This software performs the data smoothing and breakpoint recognition using a local search algorithm [28]. Based on our preliminary data from seven normal versus normal DNA hybridisations, the threshold for genetic 'gains' or 'losses' was determined as a smoothed log2 ratio $\geq +0.214$ or ≤ -0.214, respectively, corresponding to ± 2 standard deviations (data not shown). HAs or HDs were defined as a log2 ratio $\geq +0.75$ or ≤ -0.75, respectively, which corresponds to a theoretical ± 3.5-fold of the threshold for low-level alterations. All identified regions of alterations were verified by assessing the raw normalised data.

Minimal Common Regions of 'Non-Random' Genetic Alterations Identified by Multiple Sample Analysis (MSA)

The raw normalised ('non-smoothed') CGH data were analysed using the MSA software to identify minimal common regions (MCRs) of non-random genetic alterations with a statistical significance [29]. Parameter settings (http://www.cbil.upenn.edu/MSA/) used are as follows: permutations (2,000), num.tests (9), num.positions.bin (1), analyze.by (chromosomes) and significance.level ($p < 0.01$). 'Non-random' alterations were defined as genetic changes which were commonly identified in at least 14 out of 29 PDACs (≥48% of samples).

Screening for Candidate Genes Using the Pancreatic Expression Database

The transcript levels of the genes located within MCRs or regions of frequent HAs and HDs were screened using our recently constructed Pancreatic Expression Database (http://www.pancreasexpression.org/) [30]. In January 2008, there have been a total of 20 datasets of gene expression analyses deposited in the database. Each identified region was queried by setting 'GENOMIC REGION' filters; 'Chromosome', 'Start' and 'End', and its genes content was extracted if reported in PDAC or pancreatic intraepithelial neoplasia (PanIN) studies. When transcript levels of the genes within regions of aberrations were consistent with their copy number status, those genes were considered to be target candidates in the regions.

Results

Overview of Genetic Alterations Identified by aCGH-Smooth

Array CGH was performed in 29 PDAC samples and all the raw data were deposited in the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) with the accession number GSE9672. We introduced two distinct approaches to analyse our array CGH data (fig. 1). Firstly, aCGH-Smooth was used to assess the frequency of DNA copy number alterations in each sample. Figure 2 summarises genomic profiling in all 29 PDACs, and more details of these data, including published copy number variation data, are provided in the online supplementary figure 1 [31]. All the samples except PC2 have shown at least one genetic alteration and the average number of alterations per tumour was 30.2, ranging from 0 (PC2) to 66 (PC9) regions per case. Overall, genetic losses (17.9 regions per case) were more frequently observed than gains (12.3 regions per case). The most recurrent losses were observed on 9p (24 out of 29 cases; 83%), followed by 18q (22 cases; 76%), 1p, 17p (18 cases; 62%), 6q, 8p (17 cases; 59%), and 3p, 12q, Xp (16 cases; 55%). The frequent gains were detected on chromosome arms 7q, 12p, 19q (15 out of 29 cases; 52%), followed by 8q (14 cases; 48%), 1q (13 cases; 45%), 6q, 7p, 8p, (12 cases; 41%), 5q (11 cases; 38%). These findings were concordant with the previous data obtained from conventional CGH [3, 4, 32, 33].

High-Level Genetic Alterations in PDAC

We then focused on more extreme aberrations such as HAs or HDs. In total, 238 regions of HA (8.2 regions per case) were identified in 29 PDAC samples. Among those, 14 HAs were detected in at least three tumours, most of which were located on 8q (table 1). A 1.32-Mb region at 8q24.21 including the *MYC* gene was most frequently amplified (6 out of 29 cases; 21%) (online suppl. fig. 2A). Recurrent HAs (14% of cases) were also found in three other regions of 8q: 8q22.3 (618 kb), 8q24.13 (2.04 Mb) and 8q24.21-q24.22 (2.41 Mb) which contain 2, 34 and 11 annotated genes, respectively. HA at 1p21.1 (2.96 Mb) was present in 6 out of 29 cases (21%), but it was considered to be a copy number polymorphism [31].

HDs were less frequent than HAs and a total of 35 regions of HD were identified in 29 PDAC samples (1.2 regions per case). Eleven HDs were detected in at least two samples, which included some known PDAC tumour suppressor genes such as *CDKN2A* (9p21.3), *SMAD4* (18q21.2) and *DCC* (18q21.2) (table 1). The most frequent HD was observed at the locus of 9p23 (2.74 Mb in size) which includes *PTPRD* as the only known gene (3 out of 29 cases; 10%) (online supplementary fig. 2B). Two discrete regions of HD on 8p were also revealed: one region (5.33 Mb) contains the *ERICH1* gene (8p23.3), whereas the other one (1.87 Mb) includes the *TUSC3* gene (8p22).

'Non-Random' Genetic Alterations Identified by MSA

MSA has identified a total of 368 MCRs in 29 PDAC samples. Figure 3 shows MCRs on chromosome 8 and all MCRs on the entire genome are visualised in online suppl. fig. 1. Of those, 315 MCRs which consist of 115 genetic gains with a median size of 891 kb (range 175 kb–2.17 Mb) and 200 losses with a median size of 858 kb (range 107 kb–3.46 Mb) were detected in ≥48% of samples and considered to be 'non-random' (online suppl. tables 2 and 3). Notably, most MCRs were observed within regions determined by the smoothed data. Besides, the smaller size of these MCRs (68% of MCRs spanning 1 Mb or less) contributed to the reduced number of candidate genes included; on average, 12 Ensembl-annotated genes were contained in each MCR. Some MCRs included the genes known to be associated with PDAC such as *MYC* (8q24.21; 48% of samples), *KRAS* (12p12.1; 55%), *RUNX3* (1p36.11; 72%), *TP53* (17p13.1; 79%) and *DCC* (18q21.2; 48%). In addition, MSA has identified multiple novel MCRs such as gains of 5q21.1 (1.00 Mb; 90% of cases), 2p22.3 (830 kb; 86%), 5q13.3 (925 kb; 83%), 3q13.33 (904 kb; 79%), 5q33.2 (960 kb; 79%), 2q14.1 (695 kb; 76%), 16p13.3 (549 kb; 76%) and losses of 2p13.1 (1.24 Mb; 90%), 16q12.1 (1.32 Mb; 79%), 2q12.2 (981 kb; 76%), 5q31.3 (955 kb; 76%), 11p15.4 (695 kb; 76%), which were rarely detected by aCGH-Smooth (online suppl. fig. 1). These non-random alterations are commonly identified in PDAC and, therefore, are likely to have a biological significance in pancreatic carcinogenesis.

Fig. 2. Summary of genetic alterations identified by aCGH-Smooth.

Table 1. Regions of frequent high-level amplifications (HAs) and homozygous deletions (HDs) in PDAC (all the details of this table are shown in online supplementary table 1)

Cytoband	HA start bp	HA end bp	HA size Mb	Frequency of HA, %	Frequency of gain, %	Region of HA[a]		PDAC[b]		PanIN[b]	
						n	plausible candidates	n	genes	n	genes
1p21.1	102,506,927	105,468,330	2.96	21 (6/29)	21 (6/29)	8	AMY1A, AMY1B, AMY1C	1	COL11A1	0	
5p14.1	26,539,900	27,524,330	0.98	10 (3/29)	24 (7/29)	2	CDH9	0		0	
8q22.1-q22.2	96,178,048	99,215,536	3.04	10 (3/29)	21 (6/29)	25	UQCRB	1	UQCRB	0	
8q22.3	104,600,168	105,218,040	0.62	14 (4/29)	21 (6/29)	2	RIMS2	0		0	
8q23.3-q24.11	117,484,203	118,573,209	1.09	10 (3/29)	24 (7/29)	7	EIF3S3	0		0	
8q24.12-q24.13	120,863,830	122,793,256	1.93	10 (3/29)	34 (10/29)	10	COL14A1	0		0	
8q24.13	123,697,998	125,736,987	2.04	14 (4/29)	34 (10/29)	34	ZHX1	0		1	ZHX1
8q24.13-q24.21	125,736,988	128,402,048	2.67	10 (3/29)	34 (10/29)	11	SQLE	1	SQLE	1	SQLE
8q24.21	128,402,049	129,723,608	1.32	21 (6/29)	41 (12/29)	5	MYC	0		0	
8q24.21-q24.22	129,723,609	132,134,882	2.41	14 (4/29)	34 (10/29)	11	DDEF1	1	DDEF1	0	
8q24.22	132,134,883	134,190,270	2.06	10 (3/29)	34 (10/29)	11	SLA	1	SLA	0	
8q24.22	134,190,271	135,577,357	1.39	10 (3/29)	34 (10/29)	11	NDRG1	0		0	
19q12-q13.11	35,766,590	40,169,241	4.40	10 (3/29)	24 (7/29)	48	PDCD5	0		0	
19q13.12	41,059,172	42,852,411	1.79	10 (3/29)	21 (6/29)	61	ZNF461	1	ZNF461	0	
Cytoband	HD start bp	HD end bp	HD size Mb	Frequency of HD, %	Frequency of loss, %	Region of HD		PDAC		PanIN	
						n	plausible candidates	n	genes	n	genes
8p23.3-p23.2	477,644	5,805,152	5.33	7 (2/29)	52 (15/29)	16	ERICH1	1	ERICH1	1	ERICH1
8p22	14,461,155	16,333,692	1.87	7 (2/29)	52 (15/29)	5	TUSC3	0		0	
9p24.3-p24.2	190	2,136,229	2.14	7 (2/29)	79 (23/29)	16	DMRT3	0		0	
9p23	8,490,986	11,227,454	2.74	10 (3/29)	59 (17/29)	4	PTPRD	0		0	
9p21.3	20,351,133	24,090,705	3.74	7 (2/29)	59 (17/29)	32	CDKN2A, IFNB1	1	IFNB1	0	
10q23.1	82,648,711	85,603,793	2.96	7 (2/29)	17 (5/29)	6	NRG3	0		0	
12p13.31	6,100,073	7,917,137	1.82	7 (2/29)	48 (14/29)	74	SCARNA12, PEX5	2	SCARNA12, PEX5	3	ACRBP, GNB3, SPSB2
13q21.1-q21.2	55,803,709	57,629,367	1.83	7 (2/29)	34 (10/29)	9	PCDH17	0		0	
18q21.2	46,633,372	47,274,827	0.64	7 (2/29)	69 (20/29)	9	SMAD4, ME2	1	ME2	0	
18q21.2	48,496,259	50,563,150	2.07	7 (2/29)	72 (21/29)	8	DCC	0		0	
Xp22.33	2,513,592	2,619,925	0.11	7 (2/29)	48 (14/29)	1	CD99	0		0	

[a] Total number of genes and representative candidates included in each region.
[b] Genes whose transcript levels in PDAC or PanIN are consistent with their DNA copy number status identified in array CGH.

Screening for Candidate Genes Using the Pancreatic Expression Database

Using the Pancreatic Expression Database, we filtered out the genes whose expression levels are not consistent with their DNA copy number status because those genes may be incidentally co-affected along with 'true' target genes. Tables 1–3 show lists of candidate genes in which a correlation between copy numbers and transcript levels has been exhibited in PDAC. (The full details of tables 2 and 3 are seen in online supplementary tables 2 and 3, respectively.) Interestingly, *ERICH1* (8p23.3) was down-regulated in both PDACs and PanIN lesions and therefore seemed to be a possible target tumour suppressor gene in a 5.33-Mb HD region at 8p23.3-p23.2. Similarly, the database has pointed to four potential target genes (*UQCRB*, *SQLE*, *DDEF1* and *SLA*) from multiple regions of HAs on 8q, raising the possibility that these may function as oncogenes in PDAC. Although our CGH analysis has clearly delineated regions of alterations including *TUSC3* (8p22), *MYC* (8q24.21), *PTPRD* (9p23), *CDKN2A* (9p21.3), *SMAD4* (18q21.2) and *DCC* (18q21.2) genes, concordant transcript changes in these genes have not been reported in any gene expression profile deposited in the database.

The database search has also revealed a large number of possible target genes within MCRs on the entire genome (tables 2, 3). Of those, we focused on 25 candidate

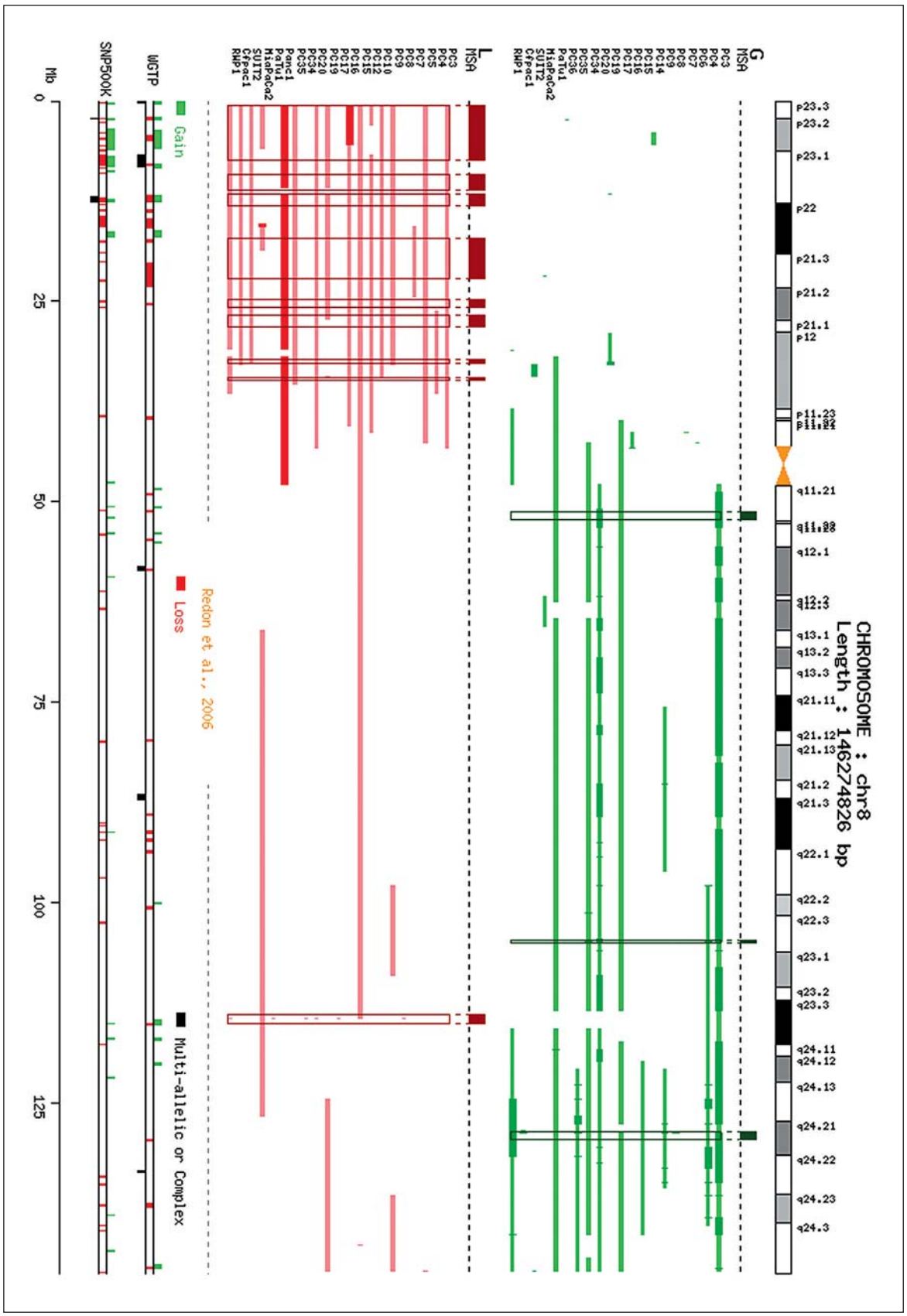
CHROMOSOME : chr8
Length : 146274826 bp
Gain
Loss
Multi-allelic or Complex
Redon et al., 2006
SNP500K
WGTP
Mb

Table 2. Minimal common regions (MCRs) of non-random genetic gains in PDAC (all the details of this table are shown in online supplementary table 2)

Cytoband	Start bp	End bp	Size Mb	Frequency %	p value	MCR[a] n	PDAC[b]		PanIN[b]	
							n	genes	n	genes
1q23.1-q23.2	156,591,077	157,602,759	1.012	76 (22/29)	0.00533	30	3	IFI16, AIM2, FCER1A	0	
2p15	61,181,674	62,979,327	1.798	69 (20/29)	0.00800	18	1	AHSA2	2	XPO1, CCT4
2q32.2	189,631,673	190,422,048	0.790	76 (22/29)	0.00267	9	2	COL5A2, ORMDL1	1	SLC40A1
3q25.32-q25.33	159,291,860	160,422,712	1.131	83 (24/29)	0.00267	9	1	RARRES1	0	
3q26.31	173,518,831	174,116,425	0.598	76 (22/29)	0.00533	15	1	AADACL1	0	
5q13.2	68,616,072	70,780,728	2.165	55 (16/29)	0.00533	33	2	BIRC1, Q59GI6_HUMAN	0	
7p22.1	5,892,806	6,067,844	0.175	55 (16/29)	0.00533	10	1	C7orf28A	1	C7orf28A
7p15.2	26,640,672	27,455,008	0.814	83 (24/29)	0.00533	16	3	HOXA3, HOXA5, HOXA11	2	SCAP2, HOXA11
10q21.1	57,203,760	58,367,659	1.164	59 (17/29)	0.00533	1	1	ZWINT	0	
10q22.1-q22.2	74,391,172	75,333,936	0.943	52 (15/29)	0.00533	27	3	SEC24C, P4HA1, USP54	0	
10q22.2	75,333,936	76,299,818	0.966	66 (19/29)	0.00267	7	1	PLAU	0	
12p12.1-p11.23	26,150,226	27,452,305	1.302	55 (16/29)	0.00800	13	2	C12orf11, ARNTL2	0	
12p11.23-p11.22	27,452,305	28,509,001	1.057	52 (15/29)	0.00800	12	1	ARNTL2	0	
15q14	32,437,506	33,877,278	1.440	62 (18/29)	0.00267	16	1	ZNF770	0	
15q26.3	99,194,139	99,788,015	0.594	66 (19/29)	0.00267	7	3	SELS_HUMAN, SNRPA1, PCSK6	0	
16p13.3	3,703,633	4,253,033	0.549	76 (22/29)	0.00267	5	1	TFAP4	1	TRAP1
16p11.2	28,085,248	29,588,307	1.503	59 (17/29)	0.00800	48	4	TUFM, SULT1A4, NR_002603.1, SPN	1	ATXN2L
16q22.1	67,951,790	68,772,637	0.821	62 (18/29)	0.00800	14	1	NQO1	0	
17q21.32	42,603,476	43,500,471	0.897	59 (17/29)	0.00267	23	1	NFE2L1	0	
18p11.32	645,198	1,339,300	0.694	48 (14/29)	0.00533	8	2	TYMS, ENOSF1	0	
19q13.43	61,736,091	62,953,519	1.217	59 (17/29)	0.00267	40	2	ZIM2, ZNF211	0	
20q11.22	32,106,257	32,792,677	0.686	62 (18/29)	0.00800	16	1	NCOA6	0	

[a] Total number of genes included in each MCR
[b] Genes whose transcript levels in PDAC or PanIN are consistent with their DNA copy number status identified in array CGH.

genes in which deregulation of transcript levels has been detected in both PDACs and PanIN lesions. Such genes are *C7orf28A* (7p22.1), *HOXA11* (7p15.2), *RERE* (1p36.23), *H6PD* (1p36.22), *ZUBR1* (1p36.13), *EYA3* (1p35.3), *SRBD1* (2p21), *TNS1* (2q35), *SLC4A4* (4q13.3), *HSPA4* (5q31.1), *PCDHB13* (5q31.3), *NUB1* (7q36.1), *ERICH1* (8p23.3), *DLC1* (8p22), *TSSC4* (11p15.5), *UEVLD* (11p15.1), *AGBL2* (11p11.2), *DSCAML1* (11q23.3), *TULP3* (12p13.33), *RASA3* (13q34), *DYNC1H1* (14q32.32), *C16orf59* (16p13.3), *SPAG7* (17p13.2), *MYH10* (17p13.1) and *CDRT4* (17p12), and these are most likely to be involved in tumour development/progression. Chromosome arm 8p has long been suspected to include critical tumour suppressor genes in PDAC, but true candidates still remain unknown [3, 33–35]. Interestingly, the database pointed to *ERICH1* and *DLC1* genes as possible tumour suppressors on multiple 8p deletions (table 3). Thus, in cases where gene expression data from the identical samples are unavailable, the database can alternatively provide useful information about transcript levels of candidate genes affected by their copy number changes based on the published data.

Fig. 3. Summary of genomic aberrations on chromosome 8 in a total of 29 PDAC samples, determined by 1-Mb-spaced CGH arrays. (The entire figure set of all chromosomes is available in online supplementary figure 1). 'Genetic gains' are shown as green bars and 'losses' as red bars according to the genomic positions (Ensembl Ver39). Thick bars are used to depict HAs and HDs. The regions surrounded by rectangles indicate all MCRs of genetic alterations identified by MSA. The regions of copy number variation were shown at the bottom [31].

Discussion

In the present study, we focused on the identification of (1) high-magnitude copy number changes and (2) 'non-random' genetic alterations in PDAC. The regions of these aberrations should be prioritised for the discovery of novel cancer-related genes because it is most likely that

Table 3. Minimal common regions (MCRs) of non-random genetic losses in PDAC (all the details of this table are shown in online supplementary table 3)

Cytoband	Start bp	End bp	Size Mb	Frequency %	p value	MCR[a] n	PDAC[b]		PanIN[b]	
							n	genes	n	genes
1p36.23	8,379,388	9,142,502	0.763	79 (23/29)	0.00267	12	1	RERE	1	RERE
1p36.23-p36.22	9,142,502	9,661,749	0.519	66 (19/29)	0.00800	8	2	H6PD, PIK3CD	1	H6PD
1p36.13	17,780,550	18,767,187	0.987	86 (25/29)	0.00267	5	1	ACTL8	0	
1p36.13	18,767,187	19,870,518	1.103	83 (24/29)	0.00267	22	1	ZUBR1	1	ZUBR1
1p36.13-p36.12	19,870,518	21,264,484	1.394	79 (23/29)	0.00267	23	1	DDOST	0	
1p36.11	23,881,482	24,596,143	0.715	76 (22/29)	0.00267	16	1	C1orf128	0	
1p36.11	26,202,341	27,453,508	1.251	76 (22/29)	0.00267	40	1	NR0B2	0	
1p36.11-p35.3	27,702,974	28,279,739	0.577	90 (26/29)	0.00267	19	1	EYA3	1	EYA3
1p13.3	109,662,726	110,421,733	0.759	76 (22/29)	0.00267	23	1	GSTM5	0	
1p13.2	111,694,621	112,653,686	0.959	76 (22/29)	0.00267	15	1	DDX20	0	
2p21	44,850,123	45,666,281	0.816	59 (17/29)	0.00800	7	1	SRBD1	1	SRBD1
2p21	46,095,190	46,912,586	0.817	66 (19/29)	0.00267	12	1	EPAS1	1	PRKCE
2q11.1	95,954,683	96,352,638	0.398	59 (17/29)	0.00800	11	1	DUSP2	1	WDR39
2q35	218,413,390	219,628,584	1.215	66 (19/29)	0.00267	39	2	TNS1, IHH	1	TNS1
3p22.2-p22.1	38,155,355	39,502,399	1.347	55 (16/29)	0.00800	28	1	AXUD1	0	
3p21.31	45,766,515	46,702,540	0.936	59 (17/29)	0.00800	19	1	CCRL2	0	
3p21.31	46,702,540	47,633,531	0.931	62 (18/29)	0.00533	20	1	PTHR1	1	CSPG5
3p21.1	52,720,543	53,444,674	0.724	79 (23/29)	0.00267	13	2	ITIH1, TKT	0	
3p14.2	61,470,834	62,712,651	1.242	66 (19/29)	0.00267	7	1	CADPS	0	
3p14.1	63,801,928	64,774,986	0.973	62 (18/29)	0.00533	9	1	PSMD6	0	
3p14.1-p13	71,243,505	72,132,566	0.889	48 (14/29)	0.00800	5	2	FOXP1, EIF4E3	0	
4q13.3	72,145,070	72,871,733	0.727	72 (21/29)	0.00533	3	1	SLC4A4	1	SLC4A4
5q31.1	131,260,927	131,590,484	0.330	59 (17/29)	0.00267	4	1	IL3	0	
5q31.1	132,157,215	134,124,694	1.967	72 (21/29)	0.00267	32	3	HSPA4, TCF7, SKP1A	2	HSPA4, CDKL3
5q31.3	140,567,235	141,522,708	0.955	76 (22/29)	0.00267	23	4	GNPDA1, PCDHGC5, PCDHB13, KIAA0141	1	PCDHB13
5q35.1-q35.2	171,457,611	172,864,129	1.407	55 (16/29)	0.00267	24	1	DUSP1	0	
6p21.32	32,547,001	33,093,794	0.547	86 (25/29)	0.00267	19	2	HLA-DQB1, HLA-DOA	1	HLA-DOA
7q21.3-q22.1	97,250,760	98,053,881	0.803	55 (16/29)	0.00267	15	1	NP_001013747.1	1	NP_001013747.1
7q36.1	150,391,833	151,096,113	0.704	69 (20/29)	0.00267	20	2	SMARCD3, NUB1_HUMAN	1	NUB1_HUMAN
8p23.3	568,026	808,128	0.240	76 (22/29)	0.00267	3	1	ERICH1	1	ERICH1
8p23.1	6,294,141	7,394,755	1.101	66 (19/29)	0.00533	38	3	DEFB1, BD02_HUMAN, DEFB104A	1	DEFB104A
8p23.1	10,310,074	11,201,017	0.891	62 (18/29)	0.00800	14	1	MTMR9	0	
8p23.1-p22	11,685,402	13,042,444	1.357	59 (17/29)	0.00800	27	1	DLC1	2	DLC1, CTSB
8p22	17,199,435	18,122,048	0.923	55 (16/29)	0.00800	9	1	FGL1	0	
8p21.3	21,304,895	22,135,548	0.831	69 (20/29)	0.00267	17	1	SFTPC	0	
8p21.2-p21.1	26,791,202	28,151,037	1.360	72 (21/29)	0.00267	18	2	CLU, CHRNA2	0	
10q22.1	72,143,312	73,316,160	1.173	59 (17/29)	0.00800	11	1	C10orf54	0	
10q22.3-q23.1	81,279,277	82,260,028	0.981	66 (19/29)	0.00267	19	1	MAT1A	0	
11p15.5	1,708,184	1,914,879	0.207	62 (18/29)	0.00267	8	2	TNNI2, LSP1	0	
11p15.5-p15.4	2,223,517	3,006,070	0.783	66 (19/29)	0.00267	20	1	TSSC4	2	TSSC4, SLC22A18AS
11p15.4	7,791,239	8,485,920	0.695	76 (22/29)	0.00267	12	1	EIF3S5	0	
11p15.2-p15.1	15,387,054	16,280,173	0.893	55 (16/29)	0.00267	1	1	SOX6	0	
11p15.1	18,386,876	19,433,497	1.047	48 (14/29)	0.00267	14	1	UEVLD	1	UEVLD
11p11.2	45,631,140	46,810,665	1.180	52 (15/29)	0.00267	23	1	ARHGAP1	0	
11p11.2	46,810,665	47,638,719	0.828	55 (16/29)	0.00267	27	1	AGBL2	1	AGBL2
11q12.1	56,639,457	57,976,581	1.337	59 (17/29)	0.00267	43	2	SLC43A1, SERPING1	0	
11q23.3	116,663,894	117,674,788	1.011	76 (22/29)	0.00267	17	3	BACE1, DSCAML1, FXYD2	1	DSCAML1
11q24.2	125,295,224	125,989,009	0.694	72 (21/29)	0.00267	14	1	SRPR	0	
12p13.33-p13.32	2,574,262	3,638,525	1.064	62 (18/29)	0.00533	22	3	TULP3, TEAD4, TSPAN9	1	TULP3
12p13.31	6,508,128	7,462,590	0.954	72 (21/29)	0.00267	44	2	SCARNA12, PEX5	3	ACRBP, GNB3, SPSB2
12q13.11	46,369,801	47,106,304	0.737	55 (16/29)	0.00800	21	2	COL2A1, SENP1	0	
12q13.13	51,376,334	51,738,002	0.362	52 (15/29)	0.00800	12	1	KRT76	0	

Table 3 (continued)

Cytoband	Start bp	End bp	Size Mb	Frequency %	p value	MCR[a] n	PDAC[b]		PanIN[b]	
							n	genes	n	genes
12q13.3	55,293,898	55,933,612	0.640	52 (15/29)	0.00800	29	1	SHMT2	0	
12q24.33	129,167,979	131,346,667	2.179	59 (17/29)	0.00800	32	1	MMP17	0	
13q34	113,314,419	113,889,199	0.575	66 (19/29)	0.00267	7	2	GAS6, RASA3	1	RASA3
14q24.2-q24.3	71,752,669	73,068,547	1.316	72 (21/29)	0.00267	17	1	NUMB	0	
14q32.12-q32.13	92,084,096	93,355,719	1.272	55 (16/29)	0.00800	14	1	CN130_HUMAN	0	
14q32.13	93,355,719	94,501,808	1.146	76 (22/29)	0.00267	21	4	SERPINA6, SERPINA4, SERPINA5, SERPINA3	0	
14q32.2	98,544,476	99,772,097	1.228	79 (23/29)	0.00267	16	1	EVL	0	
14q32.32-q32.31	99,772,097	101,675,923	1.904	69 (20/29)	0.00533	109	1	DYNC1H1	1	DYNC1H1
16p13.3	1,587,544	2,778,228	1.191	66 (19/29)	0.00533	73	3	RNPS1, NP_079384.2, NTN2L	1	NP_079384.2
16q13-q21	55,862,616	57,274,342	1.412	72 (21/29)	0.00800	38	1	CX3CL1	1	CCDC113
17p13.2	4,467,178	4,958,111	0.491	86 (25/29)	0.00267	29	3	PLD2, SLC25A11, SPAG7	1	SPAG7
17p13.1	7,151,349	7,483,027	0.332	93 (27/29)	0.00267	30	1	GPS2	0	
17p13.1	7,483,027	7,590,358	0.107	79 (23/29)	0.00267	5	1	ATP1B2	0	
17p13.1	7,590,358	8,066,706	0.476	90 (26/29)	0.00267	24	2	CHD3, GUCY2D	0	
17p13.1	8,066,706	9,024,661	0.958	72 (21/29)	0.00800	22	1	MYH10	1	MYH10
17p12-p11.2	14,985,200	16,043,971	1.059	83 (24/29)	0.00267	20	1	CDRT4	2	CDRT4, NCOR1
17p11.2	17,728,675	18,608,326	0.880	93 (27/29)	0.00267	33	1	LLGL1	0	
19p13.2-p13.13	12,385,101	13,258,496	0.873	69 (20/29)	0.00800	39	2	PRDX2, CALR	0	
21q22.3	41,614,504	45,077,643	3.463	66 (19/29)	0.00533	80	4	CU002_HUMAN, TFF3, NDUFV3, FAM3B	1	LRRC3
21q22.3	45,077,643	46,681,189	1.604	79 (23/29)	0.00267	33	2	COL6A1, COL6A2	0	
Xp22.33	2,629,805	2,868,482	0.239	86 (25/29)	0.00267	5	2	ARSD, ARSE	0	

[a] Total number of genes included in each MCR.
[b] Genes whose transcript levels in PDAC or PanIN are consistent with their DNA copy number status identified in array CGH.

candidate oncogenes or tumour suppressor genes are included in those regions [21, 22, 29, 36].

Our CGH analysis has shown that HDs are less frequent than HAs, although only purified populations of tumour cells (i.e. microdissected tissues or cell lines) were used in this study. These results indicate that 1-Mb-resolution CGH arrays are not sufficiently powerful to identify all HDs in PDAC, probably because many regions of HD may be very small [37]. For instance, this CGH study has failed to detect HD at the 9p21.3 region including *CDKN2A* and *CDKN2B* genes in PDAC tissues. However, small (45 kb) HDs at this region were identified in four tissues (PC4, 9, 15 and 18) when they were analysed by 100K single nucleotide polymorphism arrays [6]. Thus, much higher-resolution arrays are needed to fully disclose HDs in PDAC.

The smoothing algorithms such as the hidden Markov model and circular binary segmentation are now commonly used and considered to be standard statistical methods for the analysis of array CGH data [28]. However, one critical disadvantage inherent in the smoothing algorithms is a significant reduction in the native resolution of CGH arrays employed [38]. In contrast, the recently developed MSA software can be one of the alternative methods to avoid this fundamental problem and enables us to perform the highest-resolution analysis possible [29]. As a result, we have identified 315 MCRs of non-random genetic alterations in 29 PDACs, with the median size of 880 kb (<1 Mb). Most of these MCRs were observed within the broad regions defined by a smoothing algorithm, showing the utility of MSA to specify the regions where true target genes are more likely to be included. Furthermore, MSA unveiled novel additional regions of non-random aberrations which were rarely detected by aCGH-Smooth. All these results have demonstrated the high reliability and sensitivity of the algorithm used in MSA.

Thus, recent advances in analytical tools for array CGH data enable us to narrow down the regions of critical aberrations and facilitate the search for novel candidate genes. However, many of these 'narrow' regions still contained a large number of genes rather than a single

target gene. Some studies have demonstrated that the combined use of both genomic and expression profiling for the identical samples can be one of the most efficient approaches to screen for candidate genes that occur within regions of aberrations [23, 24, 39–42]. However, such a study design may not be practical for the analysis of PDAC tissues: unlike cell lines, there is substantial difficulty in obtaining a sufficient amount of high quality of mRNA from the same microdissected PDAC tissue specimens as used for DNA extraction. Alternatively, we used our Pancreatic Expression Database that allows for the rapid screening of the genes whose deregulation at the transcript level has previously been reported in PDAC and PanINs [30]. Although a large number of gene expression data for PDACs are now publicly available, it is not straightforward to extract those data for one's own research purpose due to the huge diversity of the datasets. Our database could be a powerful tool to overcome this difficulty [43, 44].

Intriguingly, the database search has pointed to four novel candidate oncogenes (*UQCRB*, *SQLE*, *DDEF1* and *SLA*) within multiple 8q amplicons. Among these, *DDEF1* is thought to be one of the prime target genes on 8q in breast, colorectal cancers and uveal melanoma, but its involvement in pancreatic carcinogenesis has not been reported [45–48]. We have also identified *ERICH1* as a novel potential target of HD event at 8p23.3, and this gene is confirmed to be down-regulated in both PDACs and PanIN lesions. Therefore, *ERICH1* might be involved in the development/progression of PDAC, although very little is known about its physiological function at present.

Similarly, the database has highlighted some candidate genes within MCRs of non-random alterations. Despite the high frequency of 8p deletion in PDAC, target candidates remain yet to be identified due to complicated patterns of genomic events occurring in this region [35]. However, our CGH study has narrowed and segmented the regions of 8p deletions, and the database has revealed the *DLC1* gene (8p22) as a potential target candidate in a 1.36-Mb MCR because its transcript is down-regulated in both PDACs and PanINs. To our knowledge, this is the first report of *DLC1* deletion in PDAC, although it has previously been reported in other types of malignancy such as liver, prostate, lung, colorectal and breast cancers [49–51]. Therefore, *DLC1*, along with *ERICH1*, could be possible tumour suppressors on 8p involved in the development/progression of PDAC.

In conclusion, we have shown that application of array CGH and the latest analytical software allows a robust and highly sensitive copy number analysis. In fact, our array CGH analysis has successfully identified the regions including some known PDAC genes such as *MYC*, *KRAS, RUNX3, CDKN2A, TP53, DPC4* and *DCC*, showing the validity of our approach. More importantly, we found many additional regions which may include critical oncogenes or tumour suppressor genes in PDAC yet to be identified. Our Pancreatic Expression Database has allowed for the rapid and cost-effective survey of transcript levels in candidate genes within those regions of aberrations. Finally, we have provided a list of novel target genes whose altered DNA copy numbers could lead to significant changes in transcript levels in PDAC. Although further validation studies are needed to prove whether their deregulated expression is universally caused by underlying copy number events, these genes are likely to have high potential as diagnostic molecular markers or therapeutic targets in PDAC.

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