RESEARCH ARTICLE

Identification of Genomic Alterations in Pancreatic Cancer Using Array-Based Comparative Genomic Hybridization

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Abstract

Background: Genomic aberration is a common feature of human cancers and also is one of the basic mechanisms that lead to overexpression of oncogenes and underexpression of tumor suppressor genes. Our study aims to identify frequent genomic changes in pancreatic cancer.

Materials and Methods: We used array comparative genomic hybridization (array CGH) to identify recurrent genomic alterations and validated the protein expression of selected genes by immunohistochemistry.

Results: Sixteen gains and thirty-two losses occurred in more than 30% and 60% of the tumors, respectively. High-level amplifications at 7q21.3–q22.1 and 19q13.2 and homozygous deletions at 1p33–p32.3, 1p22.1, 1q22, 3q27.2, 6p22.3, 6p21.31, 12q13.2, 17p13.2, 17q21.31 and 22q13.1 were identified. Especially, amplification of AKT2 was detected in two carcinomas and homozygous deletion of CDKN2C in other two cases. In 15 independent validation samples, we found that AKT2 (19q13.2) and MCM7 (7q22.1) were amplified in 6 and 9 cases, and CAMTA2 (17p13.2) and PNF1 (17p13.2) were homozygously deleted in 3 and 1 cases. AKT2 and MCM7 were overexpressed, and CAMTA2 and PNF1 were underexpressed in pancreatic cancer tissues than in morphologically normal operative margin tissues.

Both GISTIC and Genomic Workbench software identified 22q13.1 containing APOBEC3A and APOBEC3B as the only homozygous deletion region. And the expression levels of APOBEC3A and APOBEC3B were significantly lower in tumor tissues than in morphologically normal operative margin tissues. Further validation
showed that overexpression of PSCA was significantly associated with lymph node metastasis, and overexpression of HMGA2 was significantly associated with invasive depth of pancreatic cancer.

**Conclusion:** These recurrent genomic changes may be useful for revealing the mechanism of pancreatic carcinogenesis and providing candidate biomarkers.

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**Background**

Pancreatic cancer is one of the most malignant cancers in the world with a 5-year survival rate of below 5% [1]. Up to now, there is not conventional treatment with a significant impact on the course of pancreatic cancer, so that the prognosis for patients still remains poor. Therefore, identification of the molecular changes underlying this cancer will lay the foundations for improving clinical management and outcomes.

Genomic instability is a characteristic feature of almost human tumors [2]. Copy number changes are frequently found in cancers, and are believed to contribute to the initiation and progression of tumors by amplification and activation of oncogenes or deletion-induced down-expression of tumor suppressor genes. Several previous studies have identified some recurrent chromosome alterations in pancreatic cancer, such as gains on 1q, chromosomes 2, 3 and 5, 7p, 8q, 11q, 12p, 14q, 17q, 19q and 20q, losses on chromosomes 1p, 3p, 6, 8p, 9p, 10q, 13q, 14q, 15q, 17p and 18q, and amplifications of FGFR1, HER2 and DcR3 [3,4,5,6,7,8,9]. However, the available information is still limited, especially for Chinese pancreatic cancer.

The present study identified common gains, losses, amplifications and homozygous deletions in pancreatic cancer. We further evaluated the protein expression level of the copy number-increased genes HMGA2 and PSCA.

**Materials and Methods**

**Study Design**

First, the genetic aberrations in pancreatic carcinomas were detected by using Agilent 44K Human Genome CGH microarray and common genomic changes were identified. Then, we validated the protein expression of HMGA2 and PSCA which were located in the common aberration chromosome regions in pancreatic cancer.

**Patients and Samples**

Freshly resected tissues from 93 pancreatic carcinoma patients were collected by the Department of Pathology, Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China from 2006 to 2008. All the pancreatic cancer patients were
treated with radical operation, and none of them received any treatment before surgery. Representative tumor regions were excised by experienced pathologists and immediately stored at \(-70\)°C until used. All the samples used in this study were residual specimens after diagnosis sampling. Every patient signed separate informed consent forms for sampling and molecular analysis. Clinical characteristics of patients used in the array CGH study are shown in Table 1. This study was approved by the Ethics Committee of Cancer Institute and Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences (No. NCC2013B-30).

**Genomic DNA Extraction**

Genomic DNA was isolated from tumor tissues using the Qiagen DNeasy Blood & Tissue Kit as described by the manufacturer (Qiagen, Hilden, Germany). Tumor cell content of all the samples was greater than 50% by HE staining.

**Array-based CGH**

Array CGH experiments were performed using standard Agilent protocols (Agilent Technologies, Santa Clara, CA). Commercial human genomic DNA (PROMEGA, Warrington, UK) was used as reference. For each CGH hybridization, 500 ng of reference genomic DNA and the same amount of tumor DNA were digested with Alu I and RSA I restriction enzyme (PROMEGA, Warrington, UK). The digested reference DNA fragments were labeled with cyanine-3 dUTP and the tumor DNA with cyanine-5 dUTP (Agilent Technologies, Santa Clara, CA). After clean-up, reference and tumor DNA probes were mixed and hybridized onto Agilent 44K human genome CGH microarray (Agilent) for 40 h. Washing, scanning and data extraction procedures were performed following standard protocols.

**Microarray Data Analysis**

Microarray data were analyzed using Agilent Genomic Workbench (Agilent Technologies, Santa Clara, CA) and BRB-arraytools (http://linus.nci.nih.gov/BRB-ArrayTools.html). Agilent Genomic Workbench was used to calculate log\(_2\)\(^\text{ratio}\) for every probe and to identify genomic aberrations. Mean log\(_2\)\(^\text{ratio}\) of all probes in a chromosome region between 0.25 and 0.75 was classified as genomic gain, >0.75 as high-level DNA amplification, \(<-0.25\) as hemizygous loss, and \(<-0.75\) as homozygous deletion. In pathway enrichment analysis, p-value is calculated for each pathway based on the null distribution obtained by a 1000-time random sampling method.

**Real-time PCR**

The PCR reactions were performed in a total volume of 20 µl, including 10 µl of 2XPower SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK),
2 μl of cDNA/genomic DNA (5 ng/μl), and 1 μl of primer mix (10 μM each). The PCR amplification and detection were carried out in the ABI 7300 (Applied Biosystems, Warrington, UK) as follows: an initial denaturation at 95°C for 10 min; 45 cycles of 95°C for 15 s and 60°C for 1 min. The relative gene expression or relative copy number of the target gene was calculated using the comparative CT Method by normalized to an endogenous GAPDH. The relative to calibrator was given by the formula $2^{-\Delta\Delta Ct}$. $\Delta Ct$ was calculated by subtracting the average GAPDH CT from the average CT of the gene of interest. The ratio defines the level of relative expression or relative copy number of the target gene to that of GAPDH. $2^{-\Delta\Delta Ct} > 2.0$ was set for a target amplification, and $2^{-\Delta\Delta Ct} < 0.25$ was set for a target homozygous deletion.

**Immunohistochemical staining**

Formalin-fixed, paraffin-embedded pancreatic tumors were placed on the tissue microarray. For each case the cancer tissues were repeated for three times and adjacent morphologically normal tissues for two times. The slides were deparaffinized, rehydrated, immersed in 3% hydrogen peroxide solution for 10 min, heated in citrate buffer (pH 6) for 25 min at 95°C, and cooled for 60 min at room temperature. The slides were blocked by 10% normal goat serum for 30 min at 37°C and then incubated with mouse monoclonal antibody against HMGA2 (abcam, Cambridge, MA) and rabbit polyclonal antibody against PSCA (abcam, Cambridge, MA) overnight at 4°C. After being washed with PBS, the slides were incubated with biotinylated secondary antibody (diluted 1:100) for 30 min at 37°C, followed by streptavidin-peroxidase (1:100 dilution) incubation

| No. | Sex | Age | T | N | M | Grade | Pathology                |
|-----|-----|-----|---|---|---|-------|--------------------------|
| 01  | Male| 73  | 3 | 0 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 02  | Male| 60  | 3 | 1 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 03  | Male| 50  | 3 | 1 | 1 | G1    | Ductal adenocarcinoma    |
| 04  | Female | 42 | 3 | 1 | 0 | G1    | Ductal adenocarcinoma    |
| 05  | Female | 65 | 3 | 0 | 0 | G1–G2 | Ductal adenocarcinoma    |
| 06  | Male | 64  | 3 | 1 | 0 | G2    | Ductal adenocarcinoma    |
| 07  | Male | 40  | 3 | 1 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 08  | Female | 40 | 3 | 1 | 1 | G2    | Ductal adenocarcinoma    |
| 09  | Male | 73  | 3 | 1 | 0 | G3    | Ductal adenocarcinoma    |
| 10  | Male | 62  | 3 | 1 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 11  | Male | 78  | 3 | 1 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 12  | Male | 43  | 3 | 1 | 0 | G2–G3 | Ductal adenocarcinoma    |
| 13  | Male | 60  | 3 | 0 | 0 | G3    | Ductal adenocarcinoma    |
| 14  | Female | 74 | 3 | 1 | 1 | G3    | Ductal adenocarcinoma    |
| 15  | Female | 54 | 3 | 1 | 0 | G3    | Ductal adenocarcinoma    |

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for 30 min at 37°C. Immunolabeling was visualized with a mixture of 3,3’-diaminobenzidine solution. Counterstaining was carried out with hematoxylin.

Expression level was determined on the basis of staining intensity and percentage of immunoreactive cells. Negative expression (score =0) was no or faint staining, or moderate to strong staining in <25% of cells. Weak expression (score =1) was a moderate or strong staining in 25% to 50% of cells. And strong expression (score =2) was >50% of the cells with strong staining.

Statistical Analysis
Student’s t-test and Chi square test were performed with the statistical software SPSS 15.0. The differences were judged as statistically significant when the corresponding two-sided \( P \) value were <.05.

Results
Gains and Losses in Pancreatic Carcinoma Detected by Array CGH
Fifteen samples of pancreatic carcinoma were analyzed in this study and all of them had genomic changes (Range: 1 to 387). Sixteen gains and thirty-two losses were frequently detected (frequency of gain >30%, and loss >60%). The most frequent gains were 8p23.3 (41.7%), 1q44 (40%), 14q32.33 (40%), 19q13.43 (36.7%), 1q21.3 (36%) and 5q31.1–q31.2 (35.6%), and most common losses were 11p15.4 (70.7%), 15q15.1–q21.1 (70%), 3p21.31 (68.9%), 17p13.3–p13.2 (66.7%), 19p13.3–p13.2 (66.7%), 5p13.3 (63.3%), 11p11.2 (63.3%) and 19p13.3–p13.11 (63.3%). GISTIC analysis showed that copy number decrease of APOBEC3A (22q13.1) and APOBEC3B (22q13.1) was significant (Fig. 1 and Table 2).

Amplifications and Homozygous Deletions in Pancreatic Carcinoma Detected by Array CGH
High-level amplifications were detected at two chromosome regions including 7q21.3–q22.1 and 19q13.2. Homozygous deletions were identified in 1p33–p32.3, 1p22.1, 1q22, 3q27.2, 6p22.3, 6p21.31, 12q13.2, 17p13.2, 17q21.31 and 22q13.1 (Table 3). Especially, cancer gene AKT2 (19q13.2) was amplified in two carcinomas, and CDKN2C (1p33) was homozygously deleted in other two cases. (Fig. 2). By searching the COSMIC database, we found that amplification of AKT2 was associated with the increased sensitivity to the drug Z-LLN1e-CHO. More interestingly, homozygous deletion of 22q13.1 containing APOBEC3A and APOBEC3B was identified in both GISTIC and Agilent Genomic Workbench analysis (Fig. 3).

We further selected the amplified genes AKT2 (19q13.2) and MCM7 (7q22.1) and homozygous deleted genes CAMTA2 (17p13.2) and PFN1 (17p13.2) for validation by real-time PCR. In 15 independent validation samples, amplifications of AKT2 and MCM7 were detected in 6 and 9 cases, and homozygous deletions of
CAMTA2 and PFN1d in 3 and 1 cases, respectively (Fig. 4A and 4B). AKT2 and MCM7 were overexpressed, and CAMTA2 and PFN1 were underexpressed in pancreatic cancer tissues than in morphologically normal operative margin tissues (Fig. 5A and 5B).

In independent validation samples, APOBEC3A and APOBEC3B were homozygous deleted in 3 and 4 tumors, respectively (Fig. 4C). The mRNA expression levels of APOBEC3A and APOBEC3B in tumor tissues were significantly lower than in morphologically normal operative margin tissues (Fig. 5C).

Pathways Enriched for Copy Number Alterations
Pathway enrichment analysis using KEGG database was applied to the CGH data. We found that two pathways enriched in genes with gain and that six pathways
Table 2. Genomic Gains and Losses in Pancreatic Cancer.

| Change | No. | Cytoband | Start | End   | Percent1 (%) | No. of probe |
|--------|-----|----------|-------|-------|--------------|--------------|
| **Gain** |     |          |       |       |              |              |
| 1      | 8    | 8p23.3   | 181530| 1528274| 41.7         | 14           |
| 2      | 1q44 |          | 245415410| 24719291| 40.0         | 29           |
| 3      | 1q32.33 |      | 105354886| 106311914| 40.0         | 8            |
| 4      | 19q13.43 |      | 63558788 | 63784382| 36.7         | 23           |
| 5      | 1q21.3 |        | 150354126| 151576549| 36.0         | 41           |
| 6      | 5q31.1-q31.2 | | 134865707| 136298888| 35.6         | 24           |
| 7      | 2p25.3 |        | 764887 | 3196999 | 33.3         | 18           |
| 8      | 3q26.1 |        | 162699470| 168905351| 33.3         | 44           |
| 9      | 4p13-p12 |       | 42742952 | 46671044| 33.3         | 36           |
| 10     | 5p15.33-p15.31 | | 2209390 | 6426118 | 33.3         | 20           |
| 11     | 8q24.23-q24.3 | | 139224333| 140752139| 33.3         | 7            |
| 12     | 8q24.3 |        | 144974801| 145624565| 33.3         | 25           |
| 13     | 11q25 |        | 130772681| 133432246| 33.3         | 21           |
| 14     | 12p13.2 |       | 10845519| 11358635| 33.3         | 17           |
| 15     | 16q21 |        | 62462977 | 63621204 | 33.3         | 17           |
| 16     | 20q13.32-q13.33 | | 57782831| 59579107| 33.3         | 25           |
| **Loss** |     |          |       |       |              |              |
| 1      | 11p15.4 |        | 8754790 | 9967698 | 70.7         | 32           |
| 2      | 15q15.1-q21.1 | | 38644022| 42843706| 70.0         | 122          |
| 3      | 3p21.31 |        | 46978276| 49648485| 68.9         | 98           |
| 4      | 17p13.3-p13.2 | | 769430 | 5382034 | 66.7         | 173          |
| 5      | 19p13.3-p13.2 | | 2323672| 10394642| 66.7         | 322          |
| 6      | 5p13.3 |        | 32069173| 32512980| 63.3         | 16           |
| 7      | 11p11.2 |        | 46490905| 47989325| 63.3         | 57           |
| 8      | 19p13.2p13.11 | | 10432688| 19687095| 63.3         | 437          |
| 9      | 1p36.11-p32.3 | | 26563174| 51476264| 62.2         | 95           |
| 10     | 7q11.23 |        | 71858992| 75893876| 62.2         | 78           |
| 11     | 1p36.33 |        | 1698756 | 2134018 | 60.0         | 11           |
| 12     | 1q21.2-q21.3 | | 148163183| 149900117| 60.0         | 75           |
| 13     | 3p22.3 |        | 32516820| 33442286| 60.0         | 18           |
| 14     | 4p14 |        | 39145576| 40503807| 60.0         | 28           |
| 15     | 5q31.1 |        | 133588162| 133774460| 60.0         | 9            |
| 16     | 7p22.1 |        | 5831281 | 6406280 | 60.0         | 16           |
| 17     | 9q33.3 |        | 126479129| 127679820| 60.0         | 29           |
| 18     | 9q33.3-q34.13 | | 128491637| 133095053| 60.0         | 135          |
| 19     | 10q21.3 |        | 69347447| 70446758| 60.0         | 31           |
| 20     | 10q22.1 |        | 73557841| 74435066| 60.0         | 24           |
| 21     | 12p11.21 | | 31570586| 32645521| 60.0         | 16           |
| 22     | 12q24.11-q24.13 | | 108870045| 111393102| 60.0         | 67           |
| 23     | 12q24.31 |        | 121290368| 121666026| 60.0         | 12           |
| 24     | 16q21-q22.1 | | 65103378 | 69280309 | 60.0         | 183          |
| 25     | 16q22.3-q23.1 | | 72893640| 74235712| 60.0         | 45           |
| 26     | 17p13.1 |        | 6842796 | 8133829 | 60.0         | 92           |
| 27     | 17q21.2-q21.31 | | 37274288| 39139633| 60.0         | 95           |
enriched in genes with loss. The genomic gains in pancreatic carcinoma changed the pathways of gamma-hexachlorocyclohexane degradation and oxidative phosphorylation. However, cyanoamino acid metabolism, glutathione metabolism, atrazine degradation, taurine and hypotaurine metabolism, arachidonic acid

Table 2. Cont.

| Change | No. | Cytoband | Start | End | Percent1 (%) | No. of probe |
|--------|-----|----------|-------|-----|--------------|--------------|
| 28     | 19p13.3 | 529533 | 557029 | 60.0 | 2 |
| 29     | 19p13.3 | 633003 | 806290 | 60.0 | 11 |
| 30     | 19q13.12 | 40386604 | 40896554 | 60.0 | 29 |
| 31     | 19q13.12 | 41089222 | 42656912 | 60.0 | 55 |
| 32     | 22q13.2 | 39505050 | 41219454 | 60.0 | 58 |

Note: 1: when two or more adjacent cytobands have copy number changes at a frequency above 30% (gain) and 60% (loss), the average frequency of these cytobands was calculated and listed.

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Table 3. High Level Amplifications and Homozygous Deletions in Pancreatic Cancer.

| Change | No. | Cytoband | Region | Start | End | No. of cases | Gene |
|--------|-----|----------|--------|-------|-----|--------------|------|
| Amp 1  | 7q21.3–q22.1 | 97856949 | 101901147 | 2 | BAIAP2L1, NPTX2, TMEM130, TRRAP, SMURF1, KPNAA7, MYH16, ARPC1A, ARPC1B, PAPAP1, BUD31, PTC1D1, CPSF4, ATR5j2, ZNF789, ZNF394, ZKSCAN6, C7orf38, ZNF655, ZNF498, CYP3A5, CYP3A7, CYP3A4, CYP3A43, OR2A3E1, TRIM4, GJC3, AZGP1, ZKSCAN1, ZSCAN21, ZNF3, COP56, MCM7, AP4M1, TAF6, CNPY4, MBLAC1, C7orf59, C7orf43, GAL3ST4, GPC2, STAG3, GATS, PVRiG, SPYDE3, PMS2L1, PILRB, PILRA, ZCWPW1, MEPCE, C7orf47, LOC402573, TSC22DD4, C7orf51, AGF2, LRCH4, FBX024, PCOLCE, MOPD3, TFR2, ACTL6B, GN2B, GIGYF1, POP7, EPO, ZAN, EPHB4, SLC12A9, TRIP5, SRRT, UFSP1, ACHC, MUC17, TRIM56, SERPINE1, AP1S1, VGF, C7orf52, MOGAT3, PLD3, ZNHIT1, CLDN15, FLs1, RABL5, EMID2, MYL10, CUX1, SH2B2, SPYDE6, PRKRP1, ORAI2, ALKBH4, LRWD1, POLR2J |
| Amp 2  | 19q13.2 | 45178101 | 45465385 | 2 | PSMC4, ZNF546, ZNF710B, ZNF780A, MAP3K10, TTC9B, CNTD2, AKT2 |
| HD 1   | 1p33–p32.3 | 51208696 | 51476264 | 2 | CDKN2C, C1orf185, RNF11 |
| HD 2   | 1p22.1 | 93077577 | 93587765 | 2 | RPL5, SNORA66, FAM69A, MTF2, TMED5, CCDC18, DR1 |
| HD 3   | 1q22 | 154178968 | 154245532 | 2 | RXFP4, ARHGEF2, SSR2 |
| HD 4   | 3q27.2 | 187138113 | 187416929 | 2 | TRA2B, ETV5, DGKG |
| HD 5   | 6p22.3 | 16238624 | 16245913 | 3 | MYLIP |
| HD 6   | 6p21.31 | 36466570 | 36671623 | 3 | PXT1, KCTD20, STK38, SFRS3 |
| HD 7   | 12q13.2 | 54785155 | 54793934 | 2 | PA2G4, RPL41, ZC3H10 |
| HD 8   | 17p13.2 | 4789213 | 4819488 | 2 | RNF167, PFFN1, ENO3, SPAG7, CAMTA2 |
| HD 9   | 17q21.31 | 41566540 | 41624530 | 4 | KIAA1267 |
| HD 10  | 22q13.1 | 37689058 | 37715431 | 3 | APOBEC3A, APOBEC3B |

Note: Amp: amplifications. HD: homozygous deletions.

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metabolism and parkinson’s disease pathways were changed by the genomic losses (Table 4).

Validation of HMGA2 and PSCA in Pancreatic Cancer using Immunohistochemistry

Copy number increase of HMGA2 and PSCA was detected in one and four tumor, respectively. Because of their significant role in tumorigenesis [10, 11, 12, 13], we
analyzed the protein expression of HMGA2 and PSCA using immunohistochemistry (IHC). The results showed that overexpression of HMGA2 and PSCA was detected in 76.7% and 65.0% of pancreatic cancer patients, respectively (Fig. 6). Further, overexpression of PSCA was significantly associated with lymph node metastasis (Table 5), and overexpression of HMGA2 was significantly associated with invasive depth of pancreatic cancer (Table 6).

**Discussion**

Genomic aberrations can contribute to the carcinogenesis and tumor progression. In order to identify DNA copy number changes in pancreatic cancer, we performed array-based comparative genomic hybridization and found that sixteen gains with frequency above 30% and thirty-two losses above 60%, with two high-level amplifications at 7q21.3–q22.1 and 19q13.2 and ten homozygous deletions at 1p33–p32.3, 1p22.1, 1q22, 3q27.2, 6p22.3, 6p21.31, 12q13.2, 17p13.2, 17q21.31 and 22q13.1. By comparing our results with CGH data presented in progenetix
web site [14, 15], we found that most genomic aberrations were consistent. But there were still some differences. For example, loss of 9p was more frequent than loss of 9q in progenetix data, but the frequency of 9q loss was higher than 9p loss in our study. The gain of chromosome 7 was very common in progenetix data, but loss of this chromosome was more frequent in our data.

Significantly, cancer gene AKT2 was amplified in two pancreatic cancer patients, and cancer gene CDKN2C was homozygously deleted in other two cases. We validated the amplification of AKT2 and MCM7 (7q22.1) and homozygous deletion of CAMTA2 (17p13.2) and PFN1 (17p13.2) in pancreatic cancer, and further found that AKT2 and MCM7 were overexpressed, and CAMTA2 and
Fig. 5. mRNA expression of candidate genes in pancreatic cancer as compared with that in morphologically normal operative margin tissues detected by using Real-time PCR. A. Overexpression of AKT2 and MCM7. B. Underexpression of CAMTA2 and PFN1. C. Underexpression of APOBEC3A and APOBEC3B.

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Table 4. Pathways Enriched in Array CGH Data.

| Change | No. | Pathway   | Description                                | No. of genes | P value |
|--------|-----|-----------|--------------------------------------------|--------------|---------|
| Gain   | 1   | hsa00361  | gamma-Hexachlorocyclohexane degradation     | 24           | 0.001   |
|        | 2   | hsa00190  | Oxidative phosphorylation                   | 112          | 0.004   |
| Loss   | 1   | hsa00460  | Cyanoamino acid metabolism                 | 9            | 0.001   |
|        | 2   | hsa00480  | Glutathione metabolism                     | 40           | 0.001   |
|        | 3   | hsa00791  | Atrazine degradation                       | 7            | 0.001   |
|        | 4   | hsa00430  | Taurine and hypotaurine metabolism         | 10           | 0.002   |
|        | 5   | hsa00590  | Arachidonic acid metabolism                | 55           | 0.005   |
|        | 6   | hsa05020  | Parkinson’s disease                         | 15           | 0.007   |

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Fig. 6. Representative immunohistochemistry results of HMGA2 and PSCA in pancreatic cancer as compared with those in morphologically normal operative margin tissues. A. Strong and negative expression of HMGA2. B. Strong and negative expression of PSCA.

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Table 5. Association between PSCA Expression and Clinicopathological Characteristics of the Pancreatic Cancer.

| Clinical parameter | PSCA (n=58) | P value | \( \chi^2 \) | P value |
|--------------------|-------------|---------|--------------|---------|
| Age                |             |         | 1.53         | 0.216   |
| <60                | 9           | 14      |              |         |
| ≥60                | 10          | 25      |              |         |
| Sex                |             |         | 0.83         | 0.362   |
| Male               | 13          | 24      |              |         |
| Female             | 6           | 15      |              |         |
| pT                 |             |         | 5.19         | 0.075   |
| T1                 | 2           | 0       |              |         |
| T2                 | 1           | 6       |              |         |
| T3                 | 16          | 33      |              |         |
| pN                 |             |         | 4.37         | 0.037   |
| N0                 | 16          | 22      |              |         |
| N1                 | 3           | 17      |              |         |
| pM                 |             |         | 0.095        | 0.758   |
| M0                 | 16          | 34      |              |         |
| M1                 | 3           | 5       |              |         |
| Grade              |             |         | 2.029        | 0.362   |
| G1                 | 3           | 5       |              |         |
| G2                 | 6           | 20      |              |         |
| G3                 | 10          | 14      |              |         |

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PFN1 were underexpressed in pancreatic cancer as compared with those in morphologically normal operative margin tissues. These results suggested that genes including AKT2, MCM7, CAMTA2 and PFN1 might play important roles in pancreatic cancer. Homozygous deletion of CDKN2C has been found in myeloma, and copy number decrease of CDKN2C was significantly associated with a worse overall survival [16, 17, 18]. However, there was still little information about the role of CDKN2C in pancreatic cancer. Concerning the alteration of AKT2 in human malignancies, Miwa et al. have reported the amplification of AKT2 was in 3 of 12 pancreatic cancer cell lines and in 3 of 20 primary pancreatic carcinomas. Overexpression of AKT2 was also detected in the 3 cell lines with amplified AKT2 [19]. The up-regulation of AKT2 was correlated with the prognosis [20]. Tanno et al. found that active AKT promoted the invasiveness of pancreatic cancer cells through up-regulating IGF-IR expression [21]. RNAi simultaneously targeting AKT2 and K-ras could inhibit the pancreatic tumor growth [22]. Chen et al. demonstrated that AKT2 inhibition could abrogate gemcitabine-induced activation of AKT2 and NF-κB, and promote gemcitabine-induced PUMA upregulation, resulting in chemosensitization of pancreatic tumors to gemcitabine [23]. Our results further verified the amplification of AKT2 in pancreatic cancer. By searching the COSMIC database, we also found that amplification of AKT2 was associated with the increased

| Clinical parameter | HMGA2(n=60) |
|-------------------|-------------|
|                   | Negative    | Weak positive | Strong positive | χ²  | P value |
| Age               |             |              |                |     |         |
| <60               | 5           | 14           | 6              |     |         |
| ≥60               | 9           | 12           | 14             |     |         |
| Sex               |             |              |                |     |         |
| Male              | 11          | 17           | 14             |     |         |
| Female            | 3           | 9            | 6              |     |         |
| pT                |             |              |                |     |         |
| T1                | 0           | 1            | 1              |     |         |
| T2                | 2           | 3            | 2              |     |         |
| T3                | 12          | 22           | 17             |     |         |
| pN                |             |              |                |     |         |
| N0                | 10          | 21           | 6              |     |         |
| N1                | 4           | 5            | 14             |     |         |
| pM                |             |              |                |     |         |
| M0                | 10          | 23           | 17             |     |         |
| M1                | 4           | 3            | 3              |     |         |
| Grade             |             |              |                |     |         |
| G1                | 1           | 6            | 2              |     |         |
| G2                | 7           | 10           | 9              |     |         |
| G3                | 6           | 10           | 9              |     |         |

Table 6. Association between HMGA2 Expression and Clinicopathological Characteristics of the Pancreatic Cancer.

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sensitivity to the drug Z-LLNle-CHO. All these results suggested that amplification of AKT2 maybe develop into a biomarker to divide the pancreatic cancer patients into different subgroups for applying different therapy strategy. And in the future, whether the drug Z-LLNle-CHO could be used to treat the pancreatic cancer patients with AKT2 amplification should be studied.

Interestingly, both GISTIC and Genomic Workbench Software identified 22q13.1 (containing APOBEC3A and APOBEC3B) as homozygous deletion region. Real-time PCR assay showed that APOBEC3A and APOBEC3B were underexpressed in pancreatic cancer tissues than in morphologically normal operative margin tissues. APOBEC enzymes function in innate immune responses, including those that target retroviruses, suggesting links between immunity, mutagenesis and viral infection in the process of cancer development. APOBEC3A could induce hypermutation of genomic DNA and DNA double strand breaks, and catalyze the transition from a healthy to a cancer genome [24, 25]. Pham et al. reported that APOBEC3A was expressed in keratinocytes, and up-regulated in skin cancer [26]. APOBEC3B was overexpressed in a majority of ovarian cancer cell lines and high grade primary ovarian cancers. Improtantly APOBEC3B expression was correlated with total mutaion load as well as elevated levels of transversion mutations [27]. Harris et al. reported that APOBEC3B accounted for up to half of the mutational load in breast carcinomas expressing this enzyme [28]. In other cancers including bladder, cervix, lung and head and neck, APOBEC3B was also upregulated and its preferred target sequence was frequently mutated and clustered [29]. Deletion of APOBEC3B attenuated HBV clearance, and resulted in HBV infection and increased risk for developing hepatocellular carcinoma [30]. Deletion of APOBEC3 was also associated with breast cancer risk among women of European ancestry [31]. Homozygous deletion of APOBEC3B was significantly associated with unfavorable outcomes for HIV-1 acquisition and progression to AIDS [32]. It will be interesting to investigate the role of homozygous deletion of APOBEC3A and APOBEC3B in the pancreatic carcinogenesis.

HMGA2 and PSCA have been reported to be associated with pancreatic cancer. Piscuoglio et al. showed that the percentage of tumor cells with HMGA2 and HMGA1 nuclear immunoreactivity correlated positively with increasing malignancy grade and lymph node metastasis [33, 34]. And HMGA2 maintained oncogenic RAS-induced epithelial-mesenchymal transition in human pancreatic cancer cells [35]. Our study revealed that gains of HMGA2 and PSCA were detected in one and four pancreatic carcinomas, respectively. In IHC assay, overexpression of HMGA2 was detected in 76.7% and that of PSCA in 65.0% of tumors. And overexpression of PSCA was significantly associated with lymph node metastasis, and overexpression of HMGA2 was significantly associated with invasive depth of pancreatic cancer.

Overall, our study identified multiple copy number-altered chromosome regions in pancreatic cancer. These findings provide important insights into the molecular alterations associated with pancreatic tumorigenesis. Further studies
should be conducted to explore the possible tumorigenic roles of these copy number changed genes.

**Author Contributions**

Conceived and designed the experiments: MRW ZZS. Performed the experiments: JWL ZZS TYS. Analyzed the data: JWL ZZS TYS XC. Contributed reagents/materials/analysis tools: ZW SSS XX YC PZ CFW ZXZ. Wrote the paper: JWL ZZS.

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