POSTER SESSION 10: Genetics, genomics and proteomics

P10.1

The Roles of MUS81 in progression of Ovarian Cancer Associated with Dysfunctional DNA Repair Systems

Renquqn Lu[1], Suhong Xie[2], Lin Guo[2]

[1]*Fuscc, Shanghai, People's Republic of China*
[2]*Fudan University Shanghai Cancer Centeri, Shanghai, People's Republic of China*

Ovarian cancer (OC) is characterized by heterogeneity and genome instability, and has the highest mortality rate in gynecological malignancy. Novel insights into OC is required to minimize the mortality rate and drug resistant disease. MUS81, a structure -special endonucleases, plays an important role in the genome instability of cancer cells and DNA damage repair system. To investigate whether MUS81 participates in the genome instability of OC, we firstly detected the expression level of MUS81 in OC tissues (n=49) and matched adjacent cancer tissues (n=49) by RT-PCR. The result indicated that MUS81 was significantly overexpressed in OC tissues, and this data was consistent with the TCGA database. Then, the expression of MUS81 in OC cell lines was down-regulated by lentiviral-mediated RNAi, and RAPD analysis, comet assay, IFC technique etc were applied to assess the status of genome instability and DNA damage repair pathway. Interestingly, we observed that down-regulation of MUS81 in OC cells induced remarkable genome instability and decreased activity of HR, and were unable to elicit RAD51 foci formation. Furthermore, transcriptional profile analysis and interaction protein chips screening array showed that MUS81 was involved in the molecular network and pathway of DNA repair in OC cells. Further experiments proved that MUS81 had the impact on resistance to CPT and PARP inhibitors through colloboration with RAD51 and BM28, respectively. Finally, in vivo experiment also showed the evidences that down-regulation of MUS81 could increase chemotherapy-sensitivity of tumor transplanted with OC cells. Consequently, these data suggest that MUS81 might represent a novel chemotherapy target and be associated with drug resistant.

P10.3

Breast Cancer Phenotype in BRCA 1/2 carriers - preliminary analysis of three large cohorts suggests distinct subtypes based on ER status

Shai Rosenberg, Aviad Zick, Luna Kadouri, Albert Grinshpun, Amir Sonnenblick, Uziely Beatrice, Tamar Hamburger, Sari Cohen, Avital Granit, Michal Devir, Vardiella Meiner, Tamar Peretz

Hadassah Hebrew University Medical Center, Jerusalem, Israel

Background: Hereditary breast cancer (HBC) comprises more than 10% of all breast cancers (BC). BRCA1/2 genes are involved in about half of HBC. The phenotype of BRCA associated tumors differs. Most of BRCA1 associated tumors are triple negative basal-like, while BRCA2 associated tumors are mostly ER positive. In the present study we aim to further explore clinical and molecular characteristics of BRCA associated BC in 3 cohorts.
Methods: Three different BC databases (DB) were evaluated: (i) Hadassah oncogenetic BC DB (n=4429); (ii) Nick-Zainal et al. BC DB (n=560), and (iii) METABRIC BC DB (n= 1980). We tested for differences in age at diagnosis between BRCA positive (BP) and BRCA negative (BN) patients (PT), for ER positive (ER+) and ER negative (ER-) groups. Point mutations analysis was performed in cohorts ii&iii. and mRNA differential expression (DEA) and pathway analysis were performed in cohort iii, using Ingenuity Pathway Analysis (IPA).
Results: Age at diagnosis for cohorts i, ii,&iii respectively, in years for ER+:BRCA1-44, NA, 60; for BRCA2-49, 48, 64; for BN – 53, 56, 63. For ER-: BRCA1-42, 42, 47; for BRCA2-48, 52, 49; for BN-49, 54, 56. For cohorts ii&iii, higher frequencies of TP53 and PIK3Ca mutations were found among BP& BN, respectively. DEA was performed between BP&BN in ER- tumors: the major activated pathways involved cancer related processes and were highly significant (up to p=1e-7.5, FDR=1e-4.5). Surprisingly - the most significant pathway was Estrogen Mediated S-phase Entry and the most activated upstream regulator was ERBB2. Similar evaluation in ER+ showed mostly differences in immune related pathways (differences not significant).
Conclusions: Younger age at presentation was observed in BRCA1 vs. BRCA2 pt. No age differences were observed between ER+&ER- PT in cohort i&ii, in cohort iii ER- BP PT were younger than ER+ BP PT (similar age as ER+ BN). BP show different mutational profile than BN. ER+ BP and BN show similar genomic characteristics. By contrast, for ER- BP differs markedly from BN. This might imply that BP associated tumors consist of two genomically distinct subtypes: (i) ER-, and (ii) ER+ . The results may shed light on possible somatic factors which affect the development of BC BP and carry therapeutic implications.

P10.5

CBX3 promotes proliferation and aerobic glycolysis via FBW7/c-Myc pathway in pancreatic adenocarcinoma

Shuo Li, Chao Qu, Jianshan Cheng, Zhen Chen, Peng Wang, Lianyu Chen

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Introduction: Epigenetic modifications and the related chromatin modifiers are being increasingly recognized to contribute to cancer formation and progression. More and more evidence has demonstrated that CBX3 or Chromobox 3, has important role in carcinogenesis by regulating several mechanisms, such as heterochromatin formation, gene silencing and DNA replication and repair. However, its role in pancreatic adenocarcinoma (PDAC), has seldom been discussed.
Methods and Results: By using the Cancer Genome Atlas (TCGA) dataset analysis, we demonstrated that higher expression of CBX3 predicted worse prognosis. To search for the underlying molecular mechanism, we silenced CBX3 expression in PDAC cancer cell lines and identified the positive roles of CBX3 in cancer proliferation. Furthermore, we demonstrated that silencing CBX3 in pancreatic cancer cells inhibited aerobic glycolysis, the basis for providing cancer cells with building blocks for macromolecule synthesis and ATP that required. In the end, our results uncovered that CBX3 regulated aerobic glycolysis via the FBW7/c-Myc axis in pancreatic cancer.
Conclusions: These data contribute to the body of knowledge how chromatin modifiers regulated cancer malignancies and provide a critical foundation for further investigation of the role of CBX3 in malignant characteristics like proliferation, progression, and aerobic glycolysis that sustains these malignant behaviors.
Keywords: CBX3, Aerobic glycolysis, FBW7/c-Myc pathway, Pancreatic adenocarcinoma.

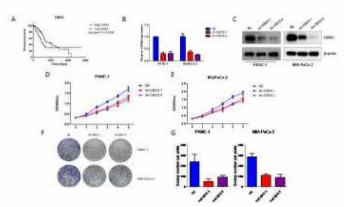
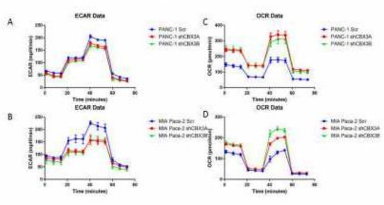


Figure1. CBX3 indicates prognosis and regulated cell proliferation and colony formation ability Data from TCGA dataset analysis indicate that higher expression of CBX3 predicted worse prognosis of pancreatic cancer (n=154, p=0.0034, log-rank test) (Figure.1A). Stably CBX3 silenced PANC-1 and MIA PaCa-2 cell lines were obtained by lentivirus mediated transfection. The efficacy was validated by quantitative PCR and western blot (Figure.1B and 1C). CBX3 knockdown inhibited cell variability as indicated by CCK-8 proliferation assay (Figure.1D and 1E). Silencing CBX3 attenuated clone formation capacity in PANC-1 and MIA PaCa-2 cells (Figure.1F and 1G).



Figur2. Abnormal cellular metabolism status induced by CBX3 knockdown CBX3 knockdown greatly inhibited ECAR, as measured by Seahorse Energy Flux analyzer (Figure.2A and 2B). OCR, usually decreased in the process of aerobic glycolysis, increased in CBX3 silenced PANC-1 and MIA PaCa-2 cells, further indicated the positive role of CBX3 in aerobic glycolysis (Figure.2C and 2D).

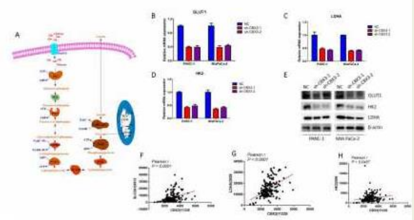


Figure.3 CBX3 positively correlated with glycolysis genes expression in vitro and in PDAC patient Schematic representation of GLUT1, HK2 and LDHA in the process of glycolysis(Figure.3A). CBX3 silencing decreased the transcriptional expression of GLUT1, HK2 and LDHA in PANC-1 and MIA PaCa-2 cells (Figure.3B-3D). CBX3 knockdown decreased the protein levels of GLUT1, HK2 and LDHA in PANC-1 and MIA PaCa-2 cells (Figure.3E). CBX3 positively correlated with expressions of GLUT1, HK2 and LDHA in the TCGA included patients respectively (Figure.3F-3H).

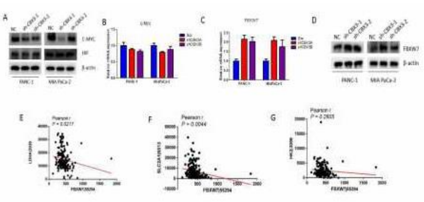


Figure.4 Dysregulation of c-MYC and FBXW7 expression by CBX3 knockdown c-MYC and HIF1α are two important regulators of aerobic glycolysis, and in CBX3 silenced PANC-1 and MIA PaCa-2 cells, c-MYC protein level decreased, while no significant changes in HIF1α were observed (Figure. 4A). CBX3 had slight impact on c-MYC in transcriptional level (Figure.4B). CBX3 negatively regulated mRNA levels of FBW7, the ubiquitin ligase that accounted for c-MYC degradation (Figure.4C). CBX3 knockdown increased FBW7 in protein levels in PANC-1 and MIA PaCa-2 cells (Figure.4D). FBW7 negatively correlated with expression of GLUT1, HK2 and LDHA in TCGA included pancreatic cancer patients (Figure.4E-4G).

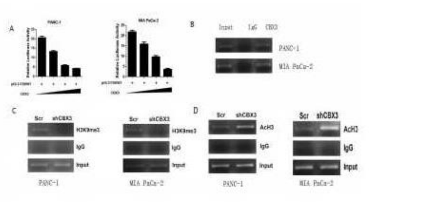


Figure.5 Epigenetic regulation of FBXW7 expression by CBX3 CBX3 inhibited FBW7 promoter luciferase activity in PANC-1 and MIA PaCa-2 cells (Figure.5A). CBX3 was enriched in CBX7 promoter region, as indicated by ChIP assay (Figure.5B). CBX3 knockdown decreased H3K9Me3 enrichment, indicated that CBX3 positively correlated with heterochromatic structure of FBW7 promoter(Figure.5C). CBX3 knockdown increased AcH3 levels in FBW7 promoter region, indicating that CBX3 silencing could loosen the heterochromatin structure of FBW7 promoter region(Figure.5D).

CBX3 promotes proliferation and aerobic glycolysis via FBW7/c-Myc pathway in pancreatic adenocarcinoma

P10.6

The molecular heterogeneity of sporadic colorectal cancer with different tumor sites in Chinese patients

Junjie Peng, Dan Huang, Sanjun Cai

Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China

Purpose: To assess the biological variability of clinical meaningful molecular markers and their clinical correlations in Chinese patients with colorectal cancer (CRC).
Materials and methods: In this prospective observational study, frequencies and clinico-pathological features of RAS and BRAFV600E mutations, deficiency of DNA mismatch repair (dMMR) were evaluated in patients with colorectal cancer staged I-IV. The molecular heterogeneity between right-sided and left-sided colorectal cancers was studied in our series by classifying patients with different mutations and dMMR status.
Results: Among 400 evaluable patients, mutations in KRAS exon 2, exon 3 or 4, NRAS and BRAFV600E were detected in 36%, 7.5%, 3.5% and 2.5%, respectively. RAS mutations were significantly higher in metastatic CRCs (56.4% vs. 43.1%, p=0.015) and right-sided CRCs (62.5% vs 41.7%, p=0.003) (Figure) . In 212 RAS wild-type patients, V600E mutation was higher in older patients (9.5% vs. 2.2%, p=0.017), women (9.2% vs. 2.2%, p=0.021) and right-sided CRCs (10.5% vs. 3.4%, p=0.06). dMMR was detected in 7.75% of all stages of CRCs, with the highest dMMR rate of 40% in stage II right-sided colon cancer.
Conclusions: By assessing the mutations and clinical correlations of RAS and BRAF genes, and dMMR status, similar RAS mutation, dMMR frequency and lower BRAF mutation was observed in Chinese patients compared to western patients. A distinct molecular heterogeneity was found between patients with right-sided and left-sided CRCs.

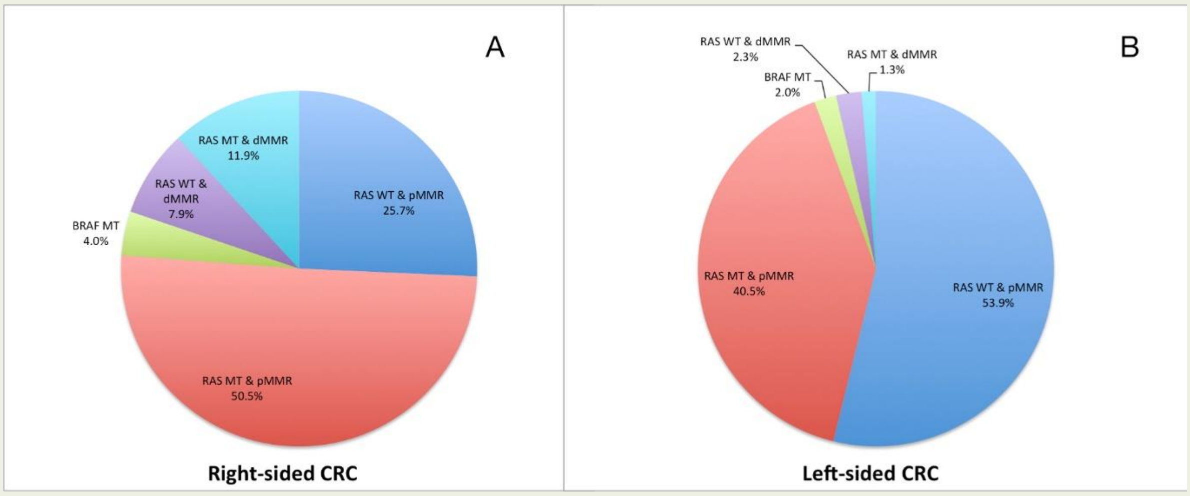


Figure 3: the molecular heterogeneity of patients with stage I-IV CRCs in right-sided (A, n=101) and left-sided (B, n=299) primary tumors.

P10.7

Genetic Evaluation of BRCA1 associated A Complex genes with Triple-negative Breast Cancer Susceptibility in Chinese Women

Hong Ling[1], Xin Hu[1], Bin Wang[2]

[1]*Fudan University Shanghai Cancer Center, Shanghai, People's Republic of China*
[2]*The University of Texas M.D. Anderson Cancer Center, Houston, TX, United States*

Background: The tumor suppressor BRCA1 plays a pivotal role in maintaining genomic stability and tumor suppression. The BRCA1-A complex is required for recruitment of BRCA1 to DNA damage sites, DNA repair and cell cycle checkpoint control. Since germline mutations of BRCA1 often lead to breast tumors that are triple-negative breast cancer (TNBC) type, we aimed to investigate whether genetic deficiency in genes of the BRCA1-A complex is associated with risk to TNBC development. Similar work has never been done before in Asian.
Methods: We investigated associations between the BRCA1-A complex genes and TNBC developing risk in the first case-control study of Chinese Han Women population including 414 patients with TNBC and 354 cancer-free controls. We detected 37 common variants in ABRAXAS, RAP80, BRE, BRCC36 and NBA1 genes encoding the BRCA1-A complex and evaluated their genetic susceptibility to the risk of TNBC. An additional cohort with 652 other types of breast cancer (non-TNBC) cases and 890 controls was used to investigate the associations between TNBC-specific SNPs genotype and non-TNBCs susceptibility. We also did in silico analysis and further function examination to the investigated SNPs.
Results: We found that rs7250266 in the promoter region of NBA1 confers a decreased risk to TNBC. The allelic frequency of the G-allele of rs7250266 was 0.19 in controls compared with 0.14 in patients with significant difference (PG) and rs2278256 (T>C) down-regulate promoter activity of NBA1 in mammary epithelial cells.
Conclusions: Genetic variants of NBA1 may be an important genetic determinant of developing TNBC. The variants detected in this study had not been reported to be associated with risk to breast cancer in literature. Further investigation and validation of these SNPs in larger cohorts may facilitate in predication and prevention of TNBC and in counseling individuals for risk of TNBC development.