EJC SUPPLEMENTS

June 2010 Volume 8, Issue 5, Page 37

140 p8 (Candidate Of Metastasis 1) drives ER-stress/autophagy/apoptosis axis induced by the synthetic cannabinoid WIN in HCC cells

O. Pellerito, P. Portanova, A. Notaro, D. Marotta, G. Calvaruso, M. Giuliano

DOI: http://dx.doi.org/10.1016/S1359-6349(10)70948-4

Abstract

Poster Sessions EJC SUPPLEMENTS 8, NO. 5 (2010) 5-81 37

accepted in clinical routine. In spite of the increasing body of high-throughput generated data, molecular tools that can help better and earlier diagnosis and set the basis for a future individualized treatment are still under development. Recently, we described Annexin A10 (ANXA10) as one of the markers included in a gene expression signature in non-muscle-invasive bladder tumours. This signature predicted both presence of concomitant CIS and progression to muscle-invasive cancer [1].
Annexins carry out biological and physiological processes including anti-coagulation, endocytosis, exocytosis, immune suppression, differentiation, tissue growth and are consistently differentially expressed in neoplasia. ANXA10 down-regulation has been correlated with poor prognosis in both hepatocellular carcinoma and gastric carcinoma [2,3].
Material and Methods: In this study, we aimed to investigate the prognostic value of ANXA10 in both non-muscle-invasive and muscle-invasive bladder cancer by immunostaining; and the function of ANXA10 following ANXA10-siRNA knock-down in bladder cancer cell lines using proliferation and wound healing techniques.
Results: Low ANXA10 nuclear staining was an independent marker for progression to muscle-invasive cancer in multivariate analysis (hazard ratio = 0.38, $P = 0.001$). In addition, low ANXA10 immunostaining in localized muscle-invasive bladder cancer ($n = 97$) was associated with development of metastatic disease ($P < 0.0000$) and short-term survival ($P < 0.0000$). The combination of ANXA10 and p53 immunostaining significantly improved the prognostic value in both non-muscle-invasive and muscle-invasive cancers. Furthermore, ANXA10 down-regulation resulted in increased cell proliferation and migration.
Conclusions: ANXA10 can be considered an independent prognostic factor for progression to muscle-invasive disease, and for development of metastatic disease in patients with muscle-invasive bladder cancer. The combination of ANXA10 expression with other potential molecular markers as e.g. p53 and RB resulted in strong predictive models of outcome. ANXA10 may identify patients with high risk of metastatic disease that may be candidates for neo-adjuvant chemotherapy.

Reference(s)
[1] Dyrskjot, L., et al., Clin Cancer Res, 2007. **13**(12): p. 3545–51.
[2] Liu, S.H., et al., Am J Pathol, 2002. **160**(5): p. 1831–7.
[3] Kim, J., et al., Int J Cancer, 2009. **125**(8): p. 1842–50.

140 p8 (Candidate Of Metastasis 1) drives ER-stress/autophagy/apoptosis axis induced by the synthetic cannabinoid WIN in HCC cells

O. Pellerito[1], P. Portanova[1], A. Notaro[1], D. Marotta[1], G. Calvaruso[1], M. Giuliano[1], [1]*Università degli Studi di Palermo, Dipartimento di Scienze Biochimiche, Palermo, Italy*

Background: Today, evidence is emerging for the role of autophagy in the regulation of life and death of tumour cells and its relationship with ER-stress signaling. Our previous results demonstrated that hepatoma HepG2 cells are sensitive to apoptotic effects induced by WIN, a synthetic cannabinoid, which acts through a mechanism involving the reduction in the levels of some survival factors and the activation of pro-apoptotic ones. Since WIN effects were observed after 36–48 hours of treatment, we investigated the possible activation of ER-stress and autophagic process in the first hours of WIN-treatment focusing our attention on p8, a factor whose expression is up-regulated in response to cannabinoid-mediated stress.
Material and Methods: ER-stress- and autophagy-related proteins were studied by RT-PCR and western blotting analysis. The autophagic morphology was estimated by MDC staining and immunofluorescence. Gene silencing was performed using small interfering RNA against p8.
Results: WIN induced ER-stress activating a pathway involving p8-CHOP-TRB3 proteins and increased the expression of the ER chaperone GRP78 which could mediate the transfer of the proapoptotic protein PAR-4 on plasma membrane. Our results indicate that WIN induced the increase in phospho-PAR-4(Thr163) level and the decrease of the pro-survival protein phospho-AKT which is responsible for an inactivating phosphorylation of PAR-4 in Ser249. Moreover, after 16h of treatment, WIN induced the appearance of autophagic vacuoles and the increase in the lipidated form of LC3 (LC3-II) which is associated with the autophagosomal membrane. The study of beclin-1 revealed a non-canonical beclin-1 independent autophagy. To evaluate the role of p8 as an activator of death pathway we carried out experiments using specific siRNA (sip8). After p8 silencing, either the markers of ER-stress (CHOP, TRB3 and GRP78) as well as those of autophagic process (LC3-II and vacuoles formation) were significantly reduced with respect to the levels observed in WIN-treated non transfected cells.
Conclusions: These findings demonstrate that ER-stress and autophagic activation are early events in WIN-induced apoptosis of HCC cells. In particular, ER-stress-related protein p8 seems to have a key role in triggering the WIN-dependent ER-stress/autophagy/apoptosis cascade in HCC cells. Moreover, the modulation of pAKT/pPAR4 balance contributes to these events.

141 Methylation profiling in non-small cell lung cancer: clinical implications

C. De Juan Chocano[1], A. Morán[1], P. Ortega[1], T. Fernández-Marcelo[1], F. Hernando[2], A. Gómez[2], J.A. López-Asenjo[3], A. Torres[2], M. Benito[1], P. Iniesta[1], [1]*Facultad de Farmacia. UCM, Bioquímica y Biología Molecular II, Madrid, Spain,* [2]*Hospital Clínico San Carlos, Cirugía, Madrid, Spain,* [3]*Hospital Príncipe de Asturias, Anatomía Patológica, Alcalá de Henares (Madrid), Spain*

Background: Lung cancer is one of the most common cancer malignancies worldwide and, according to the WHO, is the leading cause of cancer death in men and second leading cause in women. Lung cancer is unique among human solid cancers in that a single environmental factor, tobacco smoke, is believed to promote sequential changes in target cells that lead to carcinogenesis. As yet, no routine screening method that enables early detection exists, and this is a key factor in the high mortality rate of this disease. Imaging and cytology-based screening strategies have been employed for early detection, and while some are sensitive, none have been demonstrated to reduce lung cancer mortality. DNA methylation has emerged as a highly promising biomarker and is being actively studied in multiple cancers. In this work, methylation of 1505 CpG loci associated with 803 cancer-related genes were studied in forty six primary non-small lung carcinomas.
Material and Methods: Forty six primary non-small cell lung carcinomas (NSCLCs) and their corresponding control tissue samples were obtained from patients who underwent potentially curative surgery between 2000 and 2005, at San Carlos Hospital in Madrid, Spain. Illumina GoldenGate Methylation® bead array was processed according to manufacturer's protocol. Illumina BeadStudio Methylation Software was use for data analysis.
Results: Sample classification based on CpG methylation profile showed a trend towards clustering tumour versus non-tumour samples. Global hypermethylation (more than 20% of the CpG islands methylated) was associated to a worse prognosis in stage IIIA NSCLCs. In a gen-by-gene comparison of CpG methylation, twelve genes showed correlations with histological type and five with differentiation grade. More interestingly, hypermethylation of genes *CALCA* and *MMP-2* were statistically associated to a worse clinical evolution of patients), whereas hypermethylation of *RASSF1* resulted a protective variable in relation to patient prognosis. These results were independent to TNM tumour stage, as demonstrated by a Cox multivariate analysis ($P = 0.06$, $RR = 2.64$; $P = 0.03$, $RR = 2.96$; $P = 0.023$; $RR = 0.53$, respectively).
Conclusions: Global hypermethylation of a wide panel of genes may be useful as a biomarker to predict prognosis in IIIA TNM stage NSCLC. Moreover, hypermethylation in *CALCA*, *MMP-2* and *RASSF1* emerged as prognostic indicators in I-IIIA TNM stage NSCLCs, independently of tumour stage.

142 Differential expression profiles for senescence and cell death pathways in non small cell lung and colorectal tumours showing telomere shortening

T. Fernández-Marcelo[1], P. Ortega[1], A. Morán[1], C. De Juan[1], A. Sánchez-Pernaute[2], S. Hernández[3], J.R. Jarabo[2], A. Torres[2], M. Benito[1], P. Iniesta[1], [1]*Facultad de Farmacia. UCM, Bioquímica y Biología Molecular II, Madrid, Spain,* [2]*Hospital Clínico San Carlos, Cirugía, Madrid, Spain,* [3]*Hospital Clínico San Carlos, Anatomía Patológica, Madrid, Spain*

Background: Differences in how pathways of senescence and cell death operate between Non Small Cell Lung Cancer (NSCLC) and Colorectal Cancer (CRC) could explain the different clinical outcome that shortening telomere reflects, as previous results from our group showed. Our aim in this work consists of investigating whether a differential expression of factors related to these pathways could determinate differential patient outcome conferred by telomere status in NSCLC and CRC.
Material and Methods: We analyzed 36 NSCLCs, 44 CRCs, and their corresponding control tissues, obtained from patients who had undergone potentially curative surgery. Telomere function was evaluated by determining telomerase activity and telomere length. Differential expression of factors related to senescence and cell death pathways was evaluated using microarrays containing a total of 113 oligonucleotide sequences corresponding to genes from these pathways. Also, using microarrays, we investigated expression profiles of 113 genes representative of 6 biological pathways involved in transformation and tumourigenesis. We tested our results by Real Time Quantitative PCR (RT-Q-PCR).
Results: Our results indicated that 75% and 72.7% of NSCLCs and CRCs showed telomerase activity. The median telomere length was 4.15 Kb in NSCLCs and 3.8 Kb in CRCs. Microarray data indicated that NSCLCs significantly overexpressed a group of genes related to senescence and cell death pathways: *BNIP3*, *NDRG1*, *DAPK1*, *AATF*, *GADD45A* and *SHC1*, after comparing NSCLCs and CRCs with telomere attrition. *EGFR* was high and significantly overexpressed in lung tumours as compared with CRCs. Expression data from arrays were confirmed investigating gene expression by RT-Q-PCR. For NSCLCs, RT-Q-PCR analysis showed that expression levels

Article Tools

PDF (46 KB)

Email Article
Add to My Reading List
Export Citation
Create Citation Alert
Cited by in Scopus (0)

Request Permissions
Order Reprints
(100 minimum order)

Related Articles

271 POSTER Autophagy and autophagic cell death are next targets for elimination of the resistance to tyrosine kinase inhibitors
EJC Supplements, Vol. 6, Issue 12

240 INVITED Role of autophagy in cancer resistance
EJC Supplements, Vol. 6, Issue 12

Dual role of autophagy in cancer
EJC Supplements, Vol. 6, Issue 9

Hypoxia, Autophagy and Tumour Metabolism
EJC Supplements, Vol. 6, Issue 9

707 POSTER Cell death and autophagy induced by INNO-406, a novel Bcr-Abl inhibitor, in Philadelphia-positive leukaemias
EJC Supplements, Vol. 5, Issue 4

View All

June 2010 Volume 8, Issue 5, Page 37