Title (Assay)	Author	Year	Abstract	Custom staining	First author's country	Link	Citation
Cell Count							
LEDGF (p75) promotes DNA-end resection and homologous recombination	Daugaard, Mads., et al	2012	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/22773103	Nature structural & molecular biology 19.8 (2012): 803-810
Application of the Finesse glass bioreactor for fermentation of Saccharomyces cerevisiae	Perepelitsa, N., et al		See below		Switzerland	http://finesse.com/media/121717/Application_note_yeasts_V4.pdf	
Viability							
Influence of vitamin D on cisplatin sensitivity in testicular germ cell cancer-derived cell lines and in a NTera2 xenograft model	Jørgensen, A.., et al	2012	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/23098692	The Journal of steroid biochemistry and molecular biology (2012)
Proteomics Reveals that Redox Regulation Is Disrupted in Patients with Ethylmalonic Encophalopathy	Palmfeldt, J., et al.	2011	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/21410200	Journal of proteome research 10.5 (2011): 2389-2396
The influence of the number of cells scored on the sensitivity in the comet assay	Sharma, A. K., et al	2012	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/22981767	Mutation Research/Genetic Toxicology and Environmental Mutagenesis Volume 749, Issues 1–2, 12 December 2012, Pages 70–75
Sertindole, a potent antagonist at dopamine D_2 receptors, induces autophagy by increasing reactive oxygen species in SH-SY5Y neuroblastoma cells	Shin, S. Y., et al	2011	See below		South Korea	http://www.ncbi.nlm.nih.gov/pubmed/22791154	Biol Pharm Bull. 2012;35(7):1069-75
Synthesis and characterization of arginine-modified and europium-doped hydroxyapatite nanoparticle and its cell viability	Yan-zhong, z., et al	2011	See below		South Korea	http://www.sciencedirect.com/science/article/pii/S1003632611609291	Transactions of Nonferrous Metals Society of China 21.8 (2011): 1773-1778
Enzyme Induction and Cytotoxicity of Phenethyl Isothiocyanate and its Glutathione Conjugate towards Breast Cancer Cells	Lam, Y. S., and Owusu-Apenten. R	2013	See below		United Kingdom	http://eprints.ulster.ac.uk/26709/	Lam, Y. S. and Owusu-Apenten, R. (2013) Pure and Applied Chemical Sciences , 1 (2). pp. 63-73. [Journal article]
CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma	Pannem, R. R., et al	2013	See below		Sverige	http://www.ncbi.nlm.nih.gov/pubmed/24104553	Pannem, R. R., et al Carcinogenesis. 2013 Nov 8
Combining chemotherapeutic agents and netrin-1 interference potentiates cancer cell death	Paradisi, A., et al	2013	See below		France	http://onlinelibrary.wiley.com/doi/10.1002/emmm.201302654/abstract	Paradisi, A., et al EMBO Mol Med (2013) 5, 1–14
Efficient, Long Term Production of Monocyte-Derived Macrophages from Human Pluripotent Stem Cells under Partly-Defined and Fully-Defined Conditions	Wilgenburg, van B., et al	2013	See below		United Kingdom	http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0071098	Wilgenburg, van B.,et al (2013) PLoS ONE 8(8): e71098. doi:10.1371/journal.pone.0071098
Vitality							
Metallic gold beads in hyaluronic acid: a novel form of gold-based immunosuppression? Investigations of the immunosuppresive effects of metallic gold on cultured J774 macrophages and on neuronal gene exrpession in experimental autoimmune encephalomyelitis	Pedersen, D. S., et al	2013	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/23653168	BioMetals (2013): 1-17
A Novel and Rapid Apoptosis Assay Based on Thiol redox Status	Skindersoe, M. E., et al	2012	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/22407950	Cytometry Part A 81.5 (2012): 430-436
A new system for quality control in hematopoietic progenitor cell units before reinfusion in autologous transplant	Scerpa, M. C., et al	2013	See below		Italy	http://www.ncbi.nlm.nih.gov/pubmed/23789937	Transfusion. 2013 Jun 24. doi: 10.1111/trf.12307.
The octadecaneuropeptide ODN prevents 6-hydroxydopamine-induced apoptosis of cerebellar grnule neurons through a PKC-MAPK-dependent pathway	Kaddour, H., et al	2013	See below		Tunisia	http://www.ncbi.nlm.nih.gov/pubmed/23286644	J Neurochem. 2013 May;125(4):620-33. doi: 10.1111/jnc.12140. Epub 2013 Feb 19.
Cell Cycle							
Kinetics of DNA methylation inheritance by the Dnmt1-including complexes during the cell cycle	Hervouet, E., et al	2012	See below		France	http://www.celldiv.com/content/7/1/5	Cell division 7.1 (2012): 1-11
Proximity ligation in situ assay for monitoring the global DNA methylation in cells	Hervouet, E., et al	2011	See below		France	http://www.biomedcentral.com/1472-6750/11/31	BMC biotechnology 11.1 (2011): 31

Title (Assay)	Author	Year	Abstract	Custom staining	First author's country	Link	Citation
Dnmt1/Transcription Factor Interactions : An Alternative Mechanism of DNA Methylation Inheritance	Hervouet, E., et al	2010	See below		France	http://www.ncbi.nlm.nih.gov/pubmed/21779454	Genes & cancer 1.5 (2010): 434-443
Sorafenib has potent antitumor activity against multiple myeloma in vitro, ex vivo, and in vivo in the 5T33MM mouse model.	Kharaziha, P., et al	2012	See below		Sweden	http://www.ncbi.nlm.nih.gov/pubmed/22952216	Cancer research 72.20 (2012): 5348-5362
Targeting of distinct signaling cascades of cancer-associated fibroblasts define the efficacy of Sorafenib against prostate cancer cells	Kharaziha, P., et al	2012	See below		Sweden	http://www.ncbi.nlm.nih.gov/pubmed/22278289	Cell Death Dis. 2012 January; 3(1): e262.
Serum Response Factor Controls CYLD Expression via MAPK Signaling Pathway	Liang, G., et al	2011	See below		Sweden	http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.p	PloS one 6.5 (2011): e19613
MIcroRNA-15b is induced with E2F-controlled genes in HPV-related cancer	Myklebust, MP.	2011	See below		Norway	http://www.ncbi.nlm.nih.gov/pubmed/22045185	British journal of cancer 105.11 (2011): 1719-1725
Chemosensitivity Induced by Down-regulation of MicroRNA-21 in Gemcitabine-resistant Pancreatic Cancer Cells by Indole-3-Carbinol	Paik, W. H., et al	2013	See below		South Korea	http://www.ncbi.nlm.nih.gov/pubmed/23564788	Anticancer research 33.4 (2013): 1473-1481
Chromenylchalcones showing cytotoxicity on human colon cancer cell lines and in silico docking with aurora kinases	Shin, S. Y., et al	2013	See below		South Korea	http://www.sciencedirect.com/science/article/pii/S09680896130042	Bioorganic & Medicinal Chemistry Volume 21, Issue 14, 15 July 2013, Pages 4250–4258
Novel Nitrobenzazolo[3,2a]quinolinium Salts Induce Cell Death through a Mechanism Involving DNA Damage, Cell Cycle Changes, and Mitrochondrial Permeabilization	Vélez, C., et al	2013	See below		Puerto Rico	http://www.scirp.org/journal/PaperInformation.aspx?PaperID=30466	Open Journal of Apoptosis, 2013, 2, 13-22
Synthesis and Biological Evaluation of a Novel Pyrazolecarbothioamide Derivative (DK115) Inducing Cell Cycle Arrest at the G1 Phase in HCT116 Human Colon Cancer Cells	Lee, M. J., et al	2013	See below		South Korea	http://link.springer.com/article/10.1007%2Fs13765-013-3065-1	J Korean Soc Appl Biol Chem (2013) 56, 343−347
A Comparison of DNA quantitation by image and flow cytometry	Nielsen, O., et al	2011	See below		Denmark	http://chemometec.com/en-GB/Knowledge-Bank/NC-3000-vs-	
Structural Properties of Polyphenols Causing Cell Cycle Arrest at G1 Phase in HCT116 Human Colorectal Cancer Cell Lines	Shin, S. Y., et al	2013	See below		South Korea	http://www.mdpi.com/1422-0067/14/8/16970	Int. J. Mol. Sci. 2013, 14(8), 16970-16985; doi:10.3390/ijms140816970
Synthesis and Biological Evaluation of a Novel Pyrazolecarbothioamide Derivative (DK115) Inducing Cell Cycle Arrest at the G1 Phase in HCT116 Human Colon Cancer Cells	Lee, J. M., et al	2013	See below		South Korea	http://link.springer.com/article/10.1007%2Fs13765-013-3065-1	Sharma, A. K. Et al. Journal of the Korean Society for Applied Biological Chemistry June 2013, Volume 56, Issue 3, pp 343-347
Mitochondrial Potential							
Proteomics Reveals that Redox Regulation Is Disrupted in Patients with Ethylmalonic Encophalopathy	Palmfeldt, J., et al.	2011	See below		Denmark	http://www.ncbi.nlm.nih.gov/pubmed/21410200	Journal of proteome research 10.5 (2011): 2389-2396
A receptor tyrosine kinase inhibitor, Tyrphostin A9 induces cancer cell death through Drp1 dependent mitochondria fragmentation	Park, S. J., et al.	2011	See below		South Korea	http://www.ncbi.nlm.nih.gov/pubmed/21527248	Biochemical and Biophysical Research Communications 408.3 (2011): 465-470
Mitochondrial fragmentation caused by phenanthroline promotes mitophagy	Park, S. J., et al.	2012	See below		South Korea	http://www.ncbi.nlm.nih.gov/pubmed/23123158	FEBS letters Volume 586, Issue 24, 14 December 2012, p. 4303–4310
Niclosamide induces mitochondria fragmentation and promotes both apoptotic and autophagic cell death	Park, S. J., et al.	2011	See below		South Korea	http://www.ncbi.nlm.nih.gov/pubmed/21871175	Biochemistry and Molecurar Biology Reports 44.8 (2011): 517-522
A new system for quality control in hematopoietic progenitor cell units before reinfusion in autologous transplant	Scerpa, M. C., et al	2013	See below		Italy	http://www.ncbi.nlm.nih.gov/pubmed/23789937	Transfusion. 2013 Jun 24. doi: 10.1111/trf.12307.
Quantitative assesment of apoptotic events using image and flow cytometry	Nielsen, O., et al	2011	See below		Denmark	http://chemometec.com/en-GB/Knowledge-Bank/NC-3000-vs-Flow	
Annexin V							
Chromenylchalcones showing cytotoxicity on human colon cancer cell lines and in silico docking with aurora kinases	Shin, S. Y., et al	2013	See below		South Korea	ence/article/pii/S09680896130042 9X	Bioorganic & Medicinal Chemistry Volume 21, Issue 14, 15 July 2013, Pages 4250–4258

Title (Assay)	Author	Year	Abstract	Custom staining	First author's country	Link	Citation
Quantitative assesment of apoptotic events using image and flow cytometry	Nielsen, O., et al	2011	See below		Denmark	GB/Knowledge-Bank/NC-3000-vs-Flow	
Caspase							
Quantitative assesment of apoptotic events using image and flow cytometry	Nielsen, O., et al	2011	See below		Denmark	GB/Knowledge-Bank/NC-3000-vs-Flow	
DNA fragmentation							
Expression of Id proteins is regulated by the Bcl-3 proto-oncogene in prostate cancer	Ahlqvist, K., et al	2012	See below		Sweden	http://www.ncbi.nlm.nih.gov/pubmed/22580608	Oncogene Oncogene 32, 1601-1608 (21 March 2013)
Ultraviolet C Irradiation Induces Different Expression of Cyclooxygenase 2 in NIH 3T3 Cells and A431 Cells: The Roles of COX-2 Are Different in Various Cell Lines	Tai, M., et al	2012	See below		Taiwan	http://www.ncbi.nlm.nih.gov/pubmed/22605982	International Journal of Molecular Sciences13.4 (2012): 4351-436
Novel Nitrobenzazolo[3,2a]quinolinium Salts Induce Cell Death through a Mechanism Involving DNA Damage, Cell Cycle Changes, and Mitrochondrial Permeabilization	Vélez, C., et al	2013	See below		Puerto Rico	http://www.scirp.org/journal/PaperInformation.aspx?PaperID=30466	Open Journal of Apoptosis, 2013, 2, 13-22
CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma	Pannem, R. R., et al	2013	See below		Sverige	http://www.ncbi.nlm.nih.gov/pubmed/24104553	Pannem, R. R., et al Carcinogenesis. 2013 Nov 8
Flexicyte™							
Resveratrol Inhibits Alpha-Melanocyte-Stimulating Hormone Signaling, Viability, and Invasiveness in Melanoma Cells	Chen, Y., et al	2013	See below	Primary antibody: anti-c-Kit/CD117/SCFR Secondary antibody: FITC-conjugated ani-IgG TR	Taiwan	http://www.hindawi.com/journals/ecam/2013/632121/	Evidence-Based Complementary and Alternative Medicine (2013)
Secondary Coenzyme Q10 deficiency and oxidative stress in cultured fibroblasts from patients with Riboflavin Responsive Multiple Acyl-CoA Dehydrogenation Deficiency	Cornelius, N., et al	2013	See below	MitoSOXTM Red Mitochondrial Superoxide Indicator	Denmark	http://hmg.oxfordjournals.org/content/early/2013/05/31/hmg.ddt232.abstract	Human molecular genetics Volume 2013 (2013), Article ID 632121, 8 pages
Alkaline Phosphatase ALPPL-2 Is a Novel Pancreatic Carcinoma-Associated Protein	Dua, P., et al	2013	See below	Cells incubated with TAMRA-labeled SQ2 and hoechst	South Korea	http://www.ncbi.nlm.nih.gov/pubmed/23467613	Cancer Research 73.6 (2013): 1934-1945
RNA Interference-Mediated Simultaneous Silencing of Four Genes Using Cross-Shaped RNA	Lee, T. Y., et al	2013	See below	Cy3-labeled siRNA	South Korea	http://www.ncbi.nlm.nih.gov/pubmed/23563800	Molecules and cells (2013): 1-7
RNF8 and RNF168 but not HERC2 are required for DNA damage-induced ubiquitylation in chicken DT40 cells	Oestergaard, V. H., et al	2012	See below	Alexa 488/Alexa 568/Annexin V	Denmark	http://www.ncbi.nlm.nih.gov/pubmed/23010445	DNA repair (2012) 11.11: 892-905
Sunitinib and SU11652 inhibit acid sphingomyelinase, destabilize lysosomes and inhibit multidrug resistance	Ellegaard, A., et al	2013	See below	LysoTracker-Red DND-99	Denmark	http://www.ncbi.nlm.nih.gov/pubmed/23920274	

Cell Count

Nat Struct Mol Biol. 2012 Aug;19(8):803-10. doi: 10.1038/nsmb.2314. Epub 2012 Jul 8.

LEDGF (p75) promotes DNA-end resection and homologous recombination.

Daugaard M, Baude A, Fugger K, Povlsen LK, Beck H, Sørensen CS, Petersen NH, Sorensen PH, Lukas C, Bartek J, Lukas J, Rohde M, Jäättelä M.
Cell Death and Metabolism, Danish Cancer Society Research Center, Copenhagen, Denmark.

Abstract

Lens epithelium-derived growth factor p75 splice variant (LEDGF) is a chromatin-binding protein known for its antiapoptotic activity and ability to direct human immunodeficiency virus into active transcription units. Here we show that LEDGF promotes the repair of DNA double-strand breaks (DSBs) by the homologous recombination repair pathway. Depletion of LEDGF impairs the recruitment of C-terminal binding protein interacting protein (CtIP) to DNA DSBs and the subsequent CtIP-dependent DNA-end resection. LEDGF is constitutively associated with chromatin through its Pro-Trp-Trp-Pro (PWWP) domain that binds preferentially to epigenetic methyl-lysine histone markers characteristic of active transcription units. LEDGF binds CtIP in a DNA damage-dependent manner, thereby enhancing its tethering to the active chromatin and facilitating its access to DNA DSBs. These data highlight the role of PWWP-domain proteins in DNA repair and provide a molecular explanation for the antiapoptotic and cancer cell survival-activities of LEDGF.

Application of the Finesse glass bioreactor for fermentation of Saccharomyces cerevisiae.

Nadezda Perepelitsa, Stephan C. Kaiser and Dieter Eibl
Zurich University of Applied Sciences, School of Life Science and Facility Management, Institute of Biotechnology, Biochemical Engineering and Cell Cultivation Techniques, Switzerland

Abstract

This application note describes the cultivation of Saccharomyces cerevisiae cells in the Finesse glass vessel.

Viability

J Steroid Biochem Mol Biol. 2013 Jul;136:238-46. doi: 10.1016/j.jsbmb.2012.10.008. Epub 2012 Oct 23.

Influence of vitamin D on cisplatin sensitivity in testicular germ cell cancer-derived cell lines and in a NTera2 xenograft model.

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Abstract

The active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) has anti-proliferative, pro-apoptotic, and pro-differentiating effects in somatic cancer cells in vitro and in vivo. 1,25(OH)2D3 also augments the anti-tumor effects of several chemotherapeutic agents, including cisplatin, which may have clinical relevance. Given the pro-differentiation effect of vitamin D recently demonstrated in testicular germ cell tumors (TGCTs), we hypothesized that 1,25(OH)2D3 could be a beneficial adjunctive to existing chemotherapy regime used to treat these tumors. In this study, cell survival effects of 1,25(OH)2D3, another pro-differentiation compound, retinoic acid and cisplatin were investigated in TGCT-derived cell lines in vitro. 1,25(OH)2D3 augmented the effect of cisplatin in an embryonal carcinoma-derived cell line (NTera2), possibly through downregulation of pluripotency genes and simultaneous upregulation of the cell cycle regulators p21, p27, p53, p73 and FOXO1, while no significant effects were found in TCam-2 and 2102Ep cell lines (derived from seminoma and embryonal carcinoma, respectively). Anti-tumor effects of cholecalciferol, 1,25(OH)2D3, and cisplatin were subsequently tested in vivo, in a NTera2 xenograft tumor model in nude mice. In xenograft tumors, co-treatment with 1,25(OH)2D3 and cisplatin resulted in downregulation of OCT4 and simultaneous upregulation of p21 and p73, but did not reduce tumor growth significantly more than cisplatin alone. Also, cholecalciferol supplemented diet (1100IU daily) after tumor formation did not increase cisplatin sensitivity in vivo. In conclusion, addition of 1,25(OH)2D3 augmented the antitumor effect of cisplatin monotherapy in vitro, but not in this in vivo testicular germ cell cancer model. Future studies are needed to investigate potential beneficial effects of vitamin D with lower cisplatin doses, and to determine whether 1,25(OH)2D3 may increase cisplatin sensitivity in chemotherapy-resistant TGCTs. This article is part of a Special Issue entitled 'Vitamin D Workshop'.

J Proteome Res. 2011 May 6;10(5):2389-96. doi: 10.1021/pr101218d. Epub 2011 Mar 28.

Proteomics reveals that redox regulation is disrupted in patients with ethylmalonic encephalopathy.

Palmfeldt J, Vang S, Stenbroen V, Pavlou E, Baycheva M, Buchal G, Monavari AA, Augoustides-Savvopoulou P, Mandel H, Gregersen N.
Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Denmark. johan.palmfeldt@ki.au.dk

Abstract

Deficiency of the sulfide metabolizing protein ETHE1 is the cause of ethylmalonic encephalopathy (EE), an inherited and severe metabolic disorder. To study the molecular effects of EE, we performed a proteomics study on mitochondria from cultured patient fibroblast cells. Samples from six patients were analyzed and revealed seven differentially regulated proteins compared with healthy controls. Two proteins involved in pathways of detoxification and oxidative/reductive stress were underrepresented in EE patient samples: mitochondrial superoxide dismutase (SOD2) and aldehyde dehydrogenase X (ALDH1B). Sulfide:quinone oxidoreductase (SQRDL), which takes part in the same sulfide pathway as ETHE1, was also underrepresented in EE patients. The other differentially regulated proteins were apoptosis inducing factor (AIFM1), lactate dehydrogenase (LDHB), chloride intracellular channel (CLIC4) and dimethylarginine dimethylaminohydrolase 1 (DDAH1). These proteins have been reported to be involved in encephalopathy, energy metabolism, ion transport, and nitric oxide regulation, respectively. Interestingly, oxidoreductase activity was overrepresented among the regulated proteins indicating that redox perturbation plays an important role in the molecular mechanism of EE. This observation may explain the wide range of symptoms associated with the disease, and highlights the potency of the novel gaseous mediator sulfide.

Mutat Res. 2012 Dec 12;749(1-2):70-5. doi: 10.1016/j.mrgentox.2012.07.003. Epub 2012 Sep 5.

The influence of the number of cells scored on the sensitivity in the comet assay.

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Abstract

The impact on the sensitivity of the in vitro comet assay by increasing the number of cells scored has only been addressed in a few studies. The present study investigated whether the sensitivity of the assay could be improved by scoring more than 100 cells. Two cell lines and three different chemicals were used: Caco-2 cells were exposed to ethylmethane sulfonate and hydrogen peroxide in three concentrations, and HepG2 cells were exposed to ethylmethane sulfonate, hydrogen peroxide and benzo[a]pyrene in up to four concentrations, in four to five independent experiments. The scoring was carried out by means of a fully automated scoring system and the results were analyzed by evaluating the % tail DNA of 100-700 randomly selected cells for each slide consisting of two gels. By increasing the number of cells scored, the coefficients of variance decreased, leading to an improved sensitivity of the assay. A two-way ANOVA analysis of variance showed that the contribution from the two variables "the number of cells scored" and "concentration" on the total variation in the coefficients of variance dataset was statistically significant ($p<0.05$). The increase in sensitivity was demonstrated by the possibility to detect an increase in % tail DNA with statistical significance at lower concentrations. The results indicated that for low levels of DNA damage, below 9% tail DNA, scoring of 600 cells increased the sensitivity compared with scoring of 100 cells. For relatively low levels of DNA damage, about 9-16% tail DNA, scoring of 300 cells increased the sensitivity. Thus, the recommendation for the optimum number of cells scored would be 600 and 300 for low and relatively low levels of DNA damage, respectively. The findings from this study could be particularly important for bio-monitoring studies where small differences in DNA-damage levels could be relevant.

Biol Pharm Bull. 2012;35(7):1069-75.

Sertindole, a potent antagonist at dopamine D_2 receptors, induces autophagy by increasing reactive oxygen species in SH-SY5Y neuroblastoma cells.

Shin JH, Park SJ, Kim ES, Jo YK, Hong J, Cho DH.
Graduate School of East-West Medical Science, Kyung Hee University, Gyeoggi-do 446-701, Korea.

Abstract

Autophagy is associated with cell survival and cell death. Autophagy is implicated in the pathophysiology of various human diseases. In order to identify autophagy regulatory molecules, we screened a chemical drug library in SH-SY5Y cells and selected Sertindole as a potent autophagy inducer. Sertindole was developed as an antipsychotic drug for Schizophrenia. Sertindole treatment highly induced the formation of autophagosomes as well as LC3 conversion. Subsequently, Sertindole-induced autophagy was efficiently suppressed by down regulation of ATG5. Sertindole also increased reactive oxygen species (ROS) production, which contributes to autophagy-associated cell death in neuroblastoma cells. ROS scavengers such as N-acetylcysteine and Trolox suppressed not only ROS generation but also autophagy activation by Sertindole. These results suggest Sertindole induces autophagy and autophagy-associated cell death by ROS production in neuroblastoma cells.

Transactions of Nonferrous Metals Society of China, Volume 21, Issue 8, August 2011, Pages 1773–1778

Synthesis and characterization of arginine-modified and europium-doped hydroxyapatite nanoparticle and its cell viability

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[a]Medical Experiment Center in the 3rd Xiangya Hospital, Central South University, Changsha 410013, China
[b]State Key Laboratory of Powder Metallurgy, Central South University, Changsha 410083, China
[c]Research Center for Medical Material and Instruments, Central South University, Changsha 410013, China

Abstract

The arginine-modified and europium-doped hydroxyapatite nanoparticles (HAP-Eu) were synthesized by hydrothermal synthesis. The prepared nanoparticles were characterized by transmission electron microscopy (TEM), X-ray diffractometry (XRD), Fourier transform infrared (FTIR) and zeta potential analyzer. The cell viability of HAP-Eu was tested by image flow cytometry. The results indicated that HAP-Eu is short column shapes and its size is approximately 100 nm, its zeta potential is about 30.10 mV at pH of 7.5, and shows no cytotoxicity in human epithelial cells and endothelial cells.

Lam, Y. S. and Owusu-Apenten, R. (2013) Pure and Applied Chemical Sciences , 1 (2). pp. 63-73. [Journal article]

Enzyme Induction and Cytotoxicity of Phenethyl Isothiocyanate and its Glutathione Conjugate towards Breast Cancer Cells

Lam, Y. S., and Owusu-Apenten. R

Abstract

Chemoprevention using isothiocyanates is partly the result of the induction ofphase II enzymes for carcinogen detoxification from healthy cells. However,phase II enzyme activity can impair cancer therapeutic agents. The objective ofthis study was to assess phase II enzyme induction and cytotoxicity of phenethylisothiocyanate (PEITC) and glutathione conjugate with PEITC (GsPEITC) usingMCF-7 and MDA-MB-231 breast cancer cells. Changes of phase II enzymes,glutathione-S-transferase (GST) and NAD(P)H quinone reductase (QR), weremeasured by colorimetric procedures. Cell viability was determined using theMTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium- bromide) assay.From current results, 24hr exposure to <5 µM of PEITC or GsPEITC was notcytotoxic to breast cancer cells. No significant induction of phase II enzymeoccurred with a low dose range. By contrast, MCF-7 viability decreased by 50%following 24 hr treatment with 42 ±0.5 µM PEITC or 19. ±0.1 µM GsPEITC. Theconcentrations for 50% loss of viability (IC50) were 103±0.5 µM PEITC and32±0.5 µM GsPEITC in tests using MDA-MB-231 cells. Thus, MCF-7 cells weresignificantly more sensitive to PEITC or GsPEITC compared to MDA-MB-231cells. Cytotoxic doses (>50 µM) of PEITC or GsPEITC increased phase IIenzyme activity by a maximum of 500-700-percent. To conclude, cytotoxic dosesof PEITC or GsPEITC possess phase II enzyme inducing activity in MCF-7 andMDA-MB-231 breast cancer cells.

Pannem, R. R., et al Carcinogenesis. 2013 Nov 8

CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma

Pannem RR, Dorn C, Ahlqvist K, Bosserhoff AK, Hellerbrand C, Massoumi R.

Abstract

Posttranslational modification of different proteins via direct ubiquitin attachment is vital for mediating various cellular processes. Cylindromatosis (CYLD), a deubiquitination enzyme, is able to cleave the polyubiquitin chains from the substrate and to regulate different signaling pathways. Loss, or reduced expression, of CYLD is observed in different types of human cancer, such as hepatocellular carcinoma (HCC). However, the molecular mechanism by which CYLD affects cancerogenesis has to date not been unveiled. The aim of the present study was to examine how CYLD regulates cellular functions and signaling pathways during hepatocancerogenesis. We found that mice lacking CYLD were highly susceptible to chemically induced liver cancer. The mechanism behind proved to be an elevated proliferation rate of hepatocytes, owing to sustained c-Jun N-terminal kinase 1 (JNK1)-mediated signaling via ubiquitination of TNF receptor-associated factor 2 and expression of c-MYC. Overexpression of wild-type CYLD in HCC cell lines prevented cell proliferation, without affecting apoptosis, adhesion and migration. A combined immunohistochemical and tissue microarray analysis of 81 human HCC tissues revealed that CYLD expression is negatively correlated with expression of proliferation markers Ki-67 and c-MYC. To conclude, we found that downregulation of CYLD induces tumor cell proliferation, consequently contributing to the aggressive growth of HCC. Our findings suggest that CYLD holds potential to serve as a marker for HCC progression, and its link to c-MYC via JNK1 may provide the foundation for new therapeutic strategies for HCC patients.

Paradisi, A., et al EMBO Mol Med (2013) 5, 1–14

Combining chemotherapeutic agents and netrin-1 interference potentiates cancer cell death

Andrea Paradisi, Marion Creveaux, Benjamin Gibert, Guillaume Devailly, Emeline Redoulez, David Neves, Elsa Cleyssac, Isabelle Treilleux, Christian Klein, Gerhard Niederfellner, Philippe A. Cassier, Agnès Bernet, Patrick Mehlen.

Abstract

The secreted factor netrin-1 is upregulated in a fraction of human cancers as a mechanism to block apoptosis induced by netrin-1 dependence receptors DCC and UNC5H. Targeted therapies aiming to trigger tumour cell death via netrin-1/receptors interaction interference are under preclinical evaluation. We show here that Doxorubicin, 5-Fluorouracil, Paclitaxel and Cisplatin treatments trigger, in various human cancer cell lines, an increase of netrin-1 expression which is accompanied by netrin-1 receptors increase. This netrin-1 upregulation which appears to be p53-dependent is a survival mechanism as netrin-1 silencing by siRNA is associated with a potentiation of cancer cell death upon Doxorubicin treatment. We show that candidate drugs interfering with netrin-1/netrin-1 receptors interactions potentiate Doxorubicin, Cisplatin or 5-Fluorouracil-induced cancer cell death *in vitro* . Moreover, in a model of xenografted nude mice, we show that systemic Doxorubicin treatment triggers netrin-1 upregulation in the tumour but not in normal organs, enhancing and prolonging tumour growth inhibiting effect of a netrin-1 interfering drug. Together these data suggest that combining conventional chemotherapies with netrin-1 interference could be a promising therapeutic approach.

Wilgenburg, van B.,et al (2013) PLoS ONE 8(8): e71098. doi:10.1371/journal.pone.0071098

Efficient, Long Term Production of Monocyte-Derived Macrophages from Human Pluripotent Stem Cells under Partly-Defined and Fully-Defined Conditions

Bonnie van Wilgenburg, Cathy Browne, Jane Vowles, Sally A. Cowley.

Abstract

Human macrophages are specialised hosts for HIV-1, dengue virus, *Leishmania* and *Mycobacterium tuberculosis* . Yet macrophage research is hampered by lack of appropriate cell models for modelling infection by these human pathogens, because available myeloid cell lines are, by definition, not terminally differentiated like tissue macrophages. We describe here a method for deriving monocytes and macrophages from human Pluripotent Stem Cells which improves on previously published protocols in that it uses entirely defined, feeder- and serum-free culture conditions and produces very consistent, pure, high yields across both human Embryonic Stem Cell (hESC) and multiple human induced Pluripotent Stem Cell (hiPSC) lines over time periods of up to one year. Cumulatively, up to ~3×107 monocytes can be harvested per 6-well plate. The monocytes produced are most closely similar to the major blood monocyte (CD14+, CD16low, CD163+). Differentiation with M-CSF produces macrophages that are highly phagocytic, HIV-1-infectable, and upon activation produce a pro-inflammatory cytokine profile similar to blood monocyte-derived macrophages. Macrophages are notoriously hard to genetically manipulate, as they recognise foreign nucleic acids; the lentivector system described here overcomes this, as pluripotent stem cells can be relatively simply genetically manipulated for efficient transgene expression in the differentiated cells, surmounting issues of transgene silencing. Overall, the method we describe here is an efficient, effective, scalable system for the reproducible production and genetic modification of human macrophages, facilitating the interrogation of human macrophage biology.

Vitality

Biometals. 2013 Jun;26(3):369-85. doi: 10.1007/s10534-013-9616-4. Epub 2013 May 8.

Metallic gold beads in hyaluronic acid: a novel form of gold-based immunosuppression? Investigations of the immunosuppressive effects of metallic gold on cultured J774 macrophages and on neuronal gene expression in experimental autoimmune encephalomyelitis.

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Abstract

Multiple sclerosis (MS) is a neurodegenerative disease caused by recurring attacks of neuroinflammation leading to neuronal death. Immune-suppressing gold salts are used for treating connective tissue diseases; however, side effects occur from systemic spread of gold ions. This is limited by exploiting macrophage-induced liberation of gold ions (dissolucytosis) from gold surfaces. Injecting gold beads in hyaluronic acid (HA) as a vehicle into the cavities of the brain can delay clinical signs of disease progression in the MS model, experimental autoimmune encephalitis (EAE). This study investigates the anti-inflammatory properties of metallic gold/HA on the gene expression of tumor necrosis factor (Tnf-α), Interleukin (Il)-1β, Il-6, Il-10, Colony-stimulating factor (Csf)-v2, Metallothionein (Mt)-1/2, Bcl-2 associated X protein (Bax) and B cell lymphoma (Bcl)-2 in cultured J774 macrophages and in rodents with early stages of EAE. Cells grew for 5 days on gold/HA or HA, then receiving 1,000 ng/mL lipopolysaccharide (LPS) as inflammatory challenge. In the EAE experiment, 12 Lewis rats received gold injections and control groups included 11 untreated and 12 HA-treated EAE rats and five healthy animals. The experiment terminated day 9 when the first ten animals showed signs of EAE, only one of which were gold-treated (1p = 0.0367). Gene expression in the macrophages showed a statistically significant decrease in Il-6, Il-1β and Il-10-response to LPS; interestingly HA induced a statistically significant increase of Il-10. In the EAE model gene expression of inflammatory cytokines increased markedly. Compared to EAE controls levels of Tnf-α, Il-1β, Il-10, Il-6, IL-2, Ifn-γ, Il-17, transforming growth factor (Tgf)-β, superoxide dismutase (Sod)-2, Mt-2 and fibroblast growth factor (Fgf)-2 were lower in the gold-treated group. HA-treated animals expressed similar or intermediate levels. Omnibus testing for reduced inflammatory response following gold-treatment was not significant, but tendencies towards a decrease in the Sod-2, Fgf-2, Il-1β response and a higher Bdnf and IL-23 gene expression were seen. In conclusion, our findings support that bio-liberation of gold from metallic gold surfaces have anti-inflammatory properties similar to classic gold compounds, warranting further studies into the pharmacological potential of this novel gold-treatment and the possible synergistic effects of hyaluronic acid.

Cytometry A. 2012 May;81(5):430-6. doi: 10.1002/cyto.a.22032. Epub 2012 Mar 7.

A novel and rapid apoptosis assay based on thiol redox status.

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Abstract

We present here a novel probe, VitaBright-48, for the evaluation of the cellular level of reduced thiols. Using different cell lines and apoptogenic agents we show that a decrease in the level of reduced thiols correlates with well-known apoptotic markers such as phosphatidylserine translocation and caspase activity. The cell population to be investigated is added to the nonfluorescent stain VitaBright-48, which immediately permeates the cell membrane and reacts with intracellular thiols, forming a fluorescent compound. Quantification of the cell fluorescence directly after staining (without washing) can then be used to determine the population's cellular thiol level at the single cell level. Based on the results presented here, we suggest that measurement of changes in the level of free thiols should be added to the list of phenotypes which may be investigated in order to detect apoptosis.

Transfusion. 2013 Jun 24. doi: 10.1111/trf.12307.

A new system for quality control in hematopoietic progenitor cell units before reinfusion in autologous transplant.

Scerpa MC, Rossi C, Daniele N, Lanti A, Adorno G, Picardi A, Arcese W, Amadori S, Isacchi G, Zinno F.
Cryolab Center of Biotechnology and Cryobiology, Immunohematology Section, SIMT, Department of Hematology, Tor Vergata University, Rome, Italy; Rome Transplant Network, Department of Hematology, Tor Vergata University, Rome, Italy.

Abstract

BACKGROUND:

In our Center, the cell viability, the integrity of the bag, and the clonogenic assay were evaluated before the reinfusion of hematopoietic progenitor cells-apheresis (HPC-A). This quality control (QC) should be made 14 days before the reinfusion to the patient to have the result of the functional test on the proliferative capacity of hematopoietic progenitors.

STUDY DESIGN AND METHODS:

This study was designed to assess the potential of an automatic cell counting system (NucleoCounter NC-3000, ChemoMetec) in our clinical routine as a support of the clonogenic assay and the cytofluorimetric analysis for the QC of the cryopreserved HPC-A. The cell viability was evaluated by flow cytometry using the modified International Society of Hematotherapy and Graft Engineering protocol. The proliferative potential was assessed by specific clonogenic tests using a commercial medium. Furthermore, we evaluated the cellular functionality with NucleoCounter NC-3000, by using two protocols: "vitality assay" and "mitochondrial potential assay."

RESULTS:

The evaluation of the total nucleated cells in preapoptosis measured by 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazol-carbocyanine iodide (JC-1) assay showed a negative correlation ($r = -0.43$) with the total number of colonies (colony-forming unit [CFU]-granulocyte-macrophage progenitors plus burst-forming unit-erythroid progenitors plus CFU-granulocyte, erythroid, macrophage, megakaryocyte progenitors) obtained after seeding of 50×106 /L viable total nucleated cells. We observed a significant difference ($p < 0.0001$) comparing the median number of colonies (166.70; SD, ±136.36) obtained with a value of JC-1 less than 30% to the number of colonies (61.75; SD, ±59.76) obtained with a value of JC-1 more than 30%.

CONCLUSION:

The evaluation of cell functionality by the use of the NucleoCounter NC-3000 is in agreement with results from clonogenic assay and can be considered an effective alternative in the routine laboratory.

J Neurochem. 2013 May;125(4):620-33. doi: 10.1111/jnc.12140. Epub 2013 Feb 19.

The octadecaneuropeptide ODN prevents 6-hydroxydopamine-induced apoptosis of cerebellar grnule neurons through a PKC-MAPK-dependent pathway

Kaddour H, Hamdi Y, Vaudry D, Basille M, Desrues L, Leprince J, Castel H, Vaudry H, Tonon MC, Amri M, Masmoudi-Kouki O.

Abstract

Oxidative stress, induced by various neurodegenerative diseases, initiates a cascade of events leading to apoptosis, and thus plays a critical role in neuronal injury. In this study, we have investigated the potential neuroprotective effect of the octadecaneuropeptide (ODN) on 6-hydroxydopamine (6-OHDA)-induced oxidative stress and apoptosis in cerebellar granule neurons (CGN). ODN, which is produced by astrocytes, is an endogenous ligand for both central-type benzodiazepine receptors (CBR) and a metabotropic receptor. Incubation of neurons with subnanomolar concentrations of ODN (10^{-18} to 10^{-12} M) inhibited 6-OHDA-evoked cell death in a concentration-dependent manner. The effect of ODN on neuronal survival was abrogated by the metabotropic receptor antagonist, $cyclo_{1-8}$ [$DLeu^5$]OP, but not by a CBR antagonist. ODN stimulated polyphosphoinositide turnover and ERK phosphorylation in CGN. The protective effect of ODN against 6-OHDA toxicity involved the phospholipase C/ERK MAPK transduction cascade. 6-OHDA treatment induced an accumulation of reactive oxygen species, an increase of the expression of the pro-apoptotic gene Bax, a drop of the mitochondrial membrane potential and a stimulation of caspase-3 activity. Exposure of 6-OHDA-treated cells to ODN blocked all the deleterious effects of the toxin. Taken together, these data demonstrate for the first time that ODN is a neuroprotective agent that prevents 6-OHDA-induced oxidative stress and apoptotic cell death.

Cell Cycle

Cell Division 2012, 7:5 doi:10.1186/1747-1028-7-5

Kinetics of DNA methylation inheritance by the Dnmt1-including complexes during the cell cycle

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Abstract

Background

The clonal transmission of lineage-specific DNA methylation patterns in a mammalian genome during the cellular division is a crucial biological process controlled by the DNA methyltransferase Dnmt1, mainly. To investigate possible dynamic mechanisms of DNA methylation inheritance during the cell cycle, we used a Proximity Ligation In Situ Assay (P-LISA) to analyze the kinetic of formation and DNA recruitment of Dnmt1-including complexes.

Results

P-LISA, sequential chromatin immunoprecipitation and quantitative methylation specific PCR revealed that the Dnmt1/PCNA/UHRF1-including complexes are mainly formed and recruited on DNA during the S-phase of cell cycle, while the formation and the DNA recruitment of several Dnmt1/transcription factors-including complexes are not S-phase dependent but are G0/G1 and/or G2/M phases dependent.

Conclusion

Our data confirm that DNA methylation inheritance occurs in S-phase, and demonstrate that DNA methylation inheritance can also occur in G0/G1 and G2/M phases of the cell cycle.

BMC Biotechnology 2011, **11**:31 doi:10.1186/1472-6750-11-31

Proximity ligation in situ assay for monitoring the global DNA methylation in cells

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2 Université de Nantes, Faculté de Médecine, Département de Recherche en Cancérologie, IFR26, F-4400, Nantes, France

3 PICell, Plateau technique d'imagerie cellulaire - IFR26 - Centre de Recherche en Cancérologie Nantes-Angers, INSERM, U892. F-4400, Nantes, France

Abstract

Background

DNA methylation has a central role in the epigenetic control of mammalian gene expression, and is required for X inactivation, genomics imprinting and silencing of retrotransposons and repetitive sequences. Thus, several technologies have been developed to measure the degree of DNA methylation.

Results

We here present the development of the detection of protein-protein interactions via the adaptation of the proximity ligation in situ technology to evaluate the DNA methylation status in cells since the quantification of Dnmt1/PCNA interaction in cells reflects the degree of DNA methylation.

Conclusion

This method being directly realizable on cells, it appears that it could suggest a wide range of applications in basic research and drug development. More particularly, this method is specially adapted for the investigations realized from a weak quantity of biologic materiel such as stem cells or primary cultured tumor cells for examples.

Genes Cancer. 2010 May;1(5):434-43. doi: 10.1177/1947601910373794.

Dnmt1/Transcription factor interactions: an alternative mechanism of DNA methylation inheritance.

Hervouet E, Vallette FM, Cartron PF.

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Abstract

DNA methylation inheritance is the process of copying, via the DNA methyltransferase 1 (Dnmt1), the pre-existing methylation patterns onto the new DNA strand during DNA replication. Experiments of chromatin immunoprecipitation, measurement of maintenance methyltransferase activity, proximity ligation in situ assays (P-LISA, Duolink/Olink), and transcription factor arrays demonstrate that Dnmt1 interacts with transcription factors to promote site-specific DNA methylation inheritance, while the Dnmt1-PCNA-UHRF1 complex promotes the DNA methylation inheritance without site preference. We also show that the Dnmt1-PCNA-UHRF1 and Dnmt1/transcription factor complexes methylate DNA by acting as a single player or in cooperation. Thus, our data establish that the copying of the pre-existing methylation pattern is governed by the orchestration of the untargeted and the targeted mechanisms of DNA methylation inheritance, which are themselves dictated by the partners of Dnmt1.

Cancer Res. 2012 Oct 15;72(20):5348-62. doi: 10.1158/0008-5472.CAN-12-0658. Epub 2012 Sep 4.

Sorafenib has potent antitumor activity against multiple myeloma in vitro, ex vivo, and in vivo in the 5T33MM mouse model.

Kharaziha P, De Raeve H, Fristedt C, Li Q, Gruber A, Johnsson P, Kokaraki G, Panzar M, Laane E, Osterborg A, Zhivotovsky B, Jernberg-Wiklund H, Grandér D, Celsing F, Björkholm M, Vanderkerken K, Panaretakis T.

Department of Oncology-Pathology, Cancer Centre Karolinska, Karolinska University Hospital Solna, Stockholm, Sweden.

Abstract

Multiple myeloma (MM) is a B-cell malignancy characterized by the expansion of clonal plasma blasts/plasma cells within the bone marrow that relies on multiple signaling cascades, including tyrosine kinase activated pathways, to proliferate and evade cell death. Despite emerging new treatment strategies, multiple myeloma remains at present incurable. Thus, novel approaches targeting several signaling cascades by using the multi-tyrosine kinase inhibitor (TKI), sorafenib, seem a promising treatment approach for multiple myeloma. Here, we show that sorafenib induces cell death in multiple myeloma cell lines and in CD138(+)-enriched primary multiple myeloma patient samples in a caspase-dependent and -independent manner. Furthermore, sorafenib has a strong antitumoral and -angiogenic activity in the 5T33MM mouse model leading to increased overall survival. Multiple myeloma cells undergo autophagy in response to sorafenib, and inhibition of this cytoprotective pathway potentiated the efficacy of this TKI. Mcl-1, a survival factor in multiple myeloma, is downregulated at the protein level by sorafenib allowing for the execution of cell death, as ectopic overexpression of this protein protects multiple myeloma cells. Concomitant targeting of Mcl-1 by sorafenib and of Bcl-2/Bcl-xL by the antagonist ABT737 improves the efficacy of sorafenib in multiple myeloma cell lines and CD138(+)-enriched primary cells in the presence of bone marrow stromal cells. Altogether, our data support the use of sorafenib as a novel therapeutic modality against human multiple myeloma, and its efficacy may be potentiated in combination with ABT737.

Cell Death Dis. 2012 Jan 26;3:e262. doi: 10.1038/cddis.2012.1.

Targeting of distinct signaling cascades and cancer-associated fibroblasts define the efficacy of Sorafenib against prostate cancer cells.

Kharaziha P, Rodriguez P, Li Q, Rundqvist H, Björklund AC, Augsten M, Ullén A, Egevad L, Wiklund P, Nilsson S, Kroemer G, Grander D, Panaretakis T.

Department of Oncology-Pathology, Cancer Centrum Karolinska, Karolinska Institutet, Stockholm, Sweden.

Abstract

Sorafenib, a multi-tyrosine kinase inhibitor, kills more effectively the non-metastatic prostate cancer cell line 22Rv1 than the highly metastatic prostate cancer cell line PC3. In 22Rv1 cells, constitutively active STAT3 and ERK are targeted by sorafenib, contrasting with PC3 cells, in which these kinases are not active. Notably, overexpression of a constitutively active MEK construct in 22Rv1 cells stimulates the sustained phosphorylation of Bad and protects from sorafenib-induced cell death. In PC3 cells, Src and AKT are constitutively activated and targeted by sorafenib, leading to an increase in Bim protein levels. Overexpression of constitutively active AKT or knockdown of Bim protects PC3 cells from sorafenib-induced killing. In both PC3 and 22Rv1 cells, Mcl-1 depletion is required for the induction of cell death by sorafenib as transient overexpression of Mcl-1 is protective. Interestingly, co-culturing of primary cancer-associated fibroblasts (CAFs) with 22Rv1 or PC3 cells protected the cancer cells from sorafenib-induced cell death, and this protection was largely overcome by co-administration of the Bcl-2 antagonist, ABT737. In summary, the differential tyrosine kinase profile of prostate cancer cells defines the cytotoxic efficacy of sorafenib and this profile is modulated by CAFs to promote resistance. The combination of sorafenib with Bcl-2 antagonists, such as ABT737, may constitute a promising therapeutic strategy against prostate cancer.

PloS one 6.5 (2011): e19613

Serum Response Factor Controls CYLD Expression via MAPK Signaling Pathway

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Abstract

Tumor suppressor gene CYLD is a deubiquitinating enzyme which negatively regulates various signaling pathways by removing the lysine 63-linked polyubiquitin chains from several specific substrates. Loss of CYLD in different types of tumors leads to either cell survival or proliferation. In this study we demonstrate that lack of CYLD expression in CYLD−/− MEFs increases proliferation rate of these cells compared to CYLD+/+ in a serum concentration dependent manner without affecting cell survival. The reduced proliferation rate in CYLD+/+ in the presence of serum was due to the binding of serum response factor (SRF) to the serum response element identified in the CYLD promoter for the up-regulation of CYLD levels. The serum regulated recruitment of SRF to the CYLD promoter was dependent on p38 mitogen-activated protein kinase (MAPK) activity. Elimination of SRF by siRNA or inhibition of p38 MAPK reduced the expression level of CYLD and increased cell proliferation. These results show that SRF acts as a positive regulator of CYLD expression, which in turn reduces the mitogenic activation of serum for aberrant proliferation of MEF cells.

Br J Cancer. 2011 Nov 22;105(11):1719-25. doi: 10.1038/bjc.2011.457. Epub 2011 Nov 1.

MicroRNA-15b is induced with E2F-controlled genes in HPV-related cancer.

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Section of Oncology, Institute of Medicine, University of Bergen, Bergen, Norway.

Abstract

BACKGROUND

MicroRNAs (miRNAs) are important regulators of cellular processes and are found to be deregulated in many cancers. We here analysed the miRNA expression in anal carcinomas. In a previous study, we found that our anal carcinoma tumours were divided into two groups based on the expression of E2F-regulated genes. Therefore, we searched for miRNAs that could reproduce this grouping.

METHODS

A global screen of the miRNA population was performed using real-time quantitative PCR (RT-qPCR) array methods and differentially expressed miRNAs were identified. Real-time-qPCR was used to verify the expression levels of selected miRNAs and genes in a larger collection of biopsies. A siRNA-mediated knockdown of human papilloma virus (HPV)16 E7 in a cervical cell line was performed to assess the effect of E7 on miR-15b.

RESULTS

The grouping of tumours into two groups based on the expression of E2F-controlled genes was confirmed in a larger collection of anal carcinoma tumours. The expression of miR-15b was shown to be highly correlated with that of five selected E2F-induced genes (CCNA2, CCNB1, CCNB2, MSH6 and MCM7). A knockdown of HPV16 E7 resulted in decreased levels of miR-15b in Ca Ski cells.

CONCLUSION

MiR-15b expression correlates with E2F-regulated genes in anal carcinoma and appears to be part of the E2F-regulatory network.

Anticancer Res. 2013 Apr;33(4):1473-81.

Chemosensitivity induced by down-regulation of microRNA-21 in gemcitabine-resistant pancreatic cancer cells by indole-3-carbinol.

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Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea.

Abstract

BACKGROUND/AIM

Overexpression of microRNA-21 (miR-21) indicates chemoresistance in pancreatic cancer. We evaluated the change of chemosensitivity to gemcitabine through the down-regulation of miR-21 in human pancreatic cancer cells (Panc-1).

MATERIALS AND METHODS

The efficacy of indole-3-carbinol (I3C) in suppressing miR-21 expression and its anticancer effect in combination with gemcitabine were investigated.

RESULTS

Down-regulation of miR-21 by I3C was positively-correlated in a time- and dose-dependent manner. I3C and gemcitabine combination therapy increased cytotoxicity in Panc-1 cells. Transfection of miR-21 mimic abrogated I3C-induced sensitivity to gemcitabine. DNA fragmentation and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays showed that pre-treatment with I3C enhanced apoptosis and this effect was attenuated by miR-21 transfection. The expression of programmed cell death-4 (PDCD4) was increased by I3C and reduced by miR-21 transfection.

CONCLUSION

I3C would be effective for enhancing sensitivity of pancreatic cancer cells to gemcitabine via down-regulation of miR-21. Such enhanced chemosensitivity might be explained by the increased expression of PDCD4, which is a downstream target which miR-21 negatively regulates.

Bioorganic & Medicinal Chemistry - Volume 21, Issue 14, 15 July 2013, Pages 4250–4258

Chromenylchalcones showing cytotoxicity on human colon cancer cell lines and in silico docking with aurora kinases

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[b] Division of Bioscience and Biotechnology, BMIC, Konkuk University, Hwayang-Dong 1, Kwangjin-Ku, Seoul 143-701, South Korea

[c] Department of Applied Chemistry, Dongduk Women's University, Seoul 136-714, South Korea

[d] National Institute of Animal Science, Rural Development Administration, Suwon 441-706, South Korea

Abstract

Due to toxicity problems, various plant-derived compounds have been screened to find the chemotherapeutic agents. As anticancer therapeutic agents, chalcones have advantages such as poor interaction with DNA and low risk of mutagenesity. Chromenones show anticancer activities too. Therefore, hybrids of chalcone and chromenone may be potent chemotherapeutic agents. We prepared 16 synthetic chromenylchalcones and applied a clonogenic long-term survival assay method for them on HCT116 human colorectal cancer cell lines. One of chromenylchalcones tested here, chromenylchalcone 11, showed IC50 of 93.1 nM which can be competed with the IC50 values of well-known flavonoids such as catechin gallate and epicatechin gallate. Further biological experiments including cell cycle analysis, apoptosis assay, Western blot analysis, and immunofluorescent microscopy were carried out for this compound. In addition, in vitro kinases binding assay performed to explain its molecular mechanism demonstrated the compound inhibited aurora kinases. The binding modes between chromenylchalcone 11 and aurora kinases were elucidated using in silico docking experiments. These findings could be used for designing cancer therapeutic or preventive plant-derived chromenylchalcone agents.

OJApo> Vol.2 No.2, April 2013

Novel Nitrobenzazolo[3,2-a]quinolinium Salts Induce Cell Death through a Mechanism Involving DNA Damage, Cell Cycle Changes, and Mitochondrial Permeabilization

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Abstract

This study reports the capacity of three nitro substituted benzazolo[3,2-a]quinolinium salts NBQs: NBQ 95 (NSC-763304), NBQ 38 (NSC 763305), and NBQ 97 (NSC-763306) as potential antitumor agents. NBQ's are unnatural alkaloids possessing a positive charge that could facilitate interaction with cell organelles. The anticancer activities of these compounds were evaluated through the National Cancer Institute (NCI) 60 cell line screening which represents diverse histologies. The screening was performed at 10 μM on all cell lines. Results from the NCI screening indicated cytotoxicity activity on six cell lines. In order to explore a possible mechanism of action, a detailed biological activity study of NBQ 95 and NBQ 38 was performed on A431 human epidermoid carcinoma cells to determine an apoptotic pathway involving, cell cycle changes, DNA fragmentation, mutations, mitochondrial membrane permeabilization and caspases activation. DNA fragmentation, cell cycle effects, mutagenesis, mitochondrial permeabilization and activation of caspases were determined by fluorimetry and differential imaging. Our data showed that A431 growth was inhibited with an average IC50 of 30 mM. In terms of the mechanism, these compounds interacted with DNA causing fragmentation and cell cycle arrest at sub G0/G1 stage. Mutagenesis was higher for NBQ 38 and moderate for NBQ 95 Mitochondrial permeabilization was observed with NBQ 38 and slightly for NBQ 95. Both compounds caused activation of Caspases 3 and 7 suggesting an apoptotic cell death pathway through an intrinsic mechanism. This study reports evidence of the toxicity of these novel compounds with overlapping structural and mechanistic similarities to ellipticine, a known anti-tumor compound.

Journal of the Korean Society for Applied Biological Chemistry - June 2013, Volume 56, Issue 3, pp 343-347

Synthesis and biological evaluation of a novel pyrazolecarbothioamide derivative (DK115) inducing cell cycle arrest at the G1 phase in HCT116 human colon cancer cells

Jong Min Lee, Soon Young Shin, Hyuk Yoon, Mi So Lee, You Ri Lee, Dongsoo Koh, Young Han Lee

Abstract

A novel compound, 5-(2,3-dimethoxyphenyl)-3-(1-hydroxynaphthalen-2-yl)-N-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (designated DK115) was synthesized, and its antitumor efficacy was assessed. Exposure of DK115 to HCT116 human colon cancer cells

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A comparison of DNA quantitation by image and flow cytometry

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Abstract

The most common approach for determining the cell cycle stage is based on quantification of cellular DNA content. DNA content can be determined using fluorescent DNA-selective stains that exhibit emission signals proportional to DNA mass. DNA staining is typically performed on cells permeabilzed with either nonionic detegents or alcohol fixation. Traditionally, flow cytometry has been the method of choice for analysing cell cycle distribution and, currently, stands as the gold standard. We have employed an image based system, NucleoCounter NC-3000, for quantifying DNA content of different mammalian cell lines stained with DAPI. NC-3000 demonstrated accurate and precise determination of cell cycle stages compared to flow cytometric analyses (BD LSRII).

Structural Properties of Polyphenols Causing Cell Cycle Arrest at G1 Phase in HCT116 Human Colorectal Cancer Cell Lines

Soon Young Shin[1], Hyuk Yoon[2], Seunghyun Ahn[3], Dong-Wook Kim[4], Dong-Ho Bae[2], Dongsoo Koh[3], Young Han Lee[1] and Yoongho Lim[2]

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[3]Department of Applied Chemistry, Dongduk Women's University, Seoul 136-714, Korea

[4]National Institute of Animal Science, Rural Development Administration, Suwon 441-706, Korea

Abstract

Plant-derived polyphenols are being tested as chemopreventive agents; some polyphenols arrest the cell cycle at G1 phase, whereas others inhibit cell cycle proliferation at G2/M phase. Therefore, polyphenols have been proposed to inhibit cell cycle progression at different phases via distinct mechanisms. Indeed, our previous studies showed that small structural differences in polyphenols cause large differences in their biological activities; however, the details of the structural properties causing G1 cell cycle arrest remain unknown. In this study, we prepared 27 polyphenols, including eight different scaffolds, to gain insight into the structural conditions that arrest the cell cycle at G1 phase in a quantitative structure–activity relationship study. We used cell cycle profiles to determine the biophores responsible for G1 cell cycle arrest and believe that the biophores identified in this study will help design polyphenols that cause G1 cell cycle arrest.

Sharma, A. K. Et al. Journal of the Korean Society for Applied Biological Chemistry June 2013, Volume 56, Issue 3, pp 343-347

Synthesis and Biological Evaluation of a Novel Pyrazolecarbothioamide Derivative (DK115) Inducing Cell Cycle Arrest at the G1 Phase in HCT116 Human Colon Cancer Cells

Jong Min Lee, Soon Young Shin, Hyuk Yoon, Mi So Lee, You Ri Lee, Dongsoo Koh, Young Han Lee.

Abstract

A novel compound, 5-(2,3-dimethoxyphenyl)-3-(1-hydroxynaphthalen-2-yl)-*N* -(4-methoxyphenyl)-4,5-dihydro-1*H* -pyrazole-1-carbothioamide (designated DK115) was synthesized, and its antitumor efficacy was assessed. Exposure of DK115 to HCT116 human colon cancer cells inhibited cellular proliferation and clonogenicity. DK115 induced cell cycle arrest at the G1 phase. DK115 downregulated cyclin D1 expression, whereas upregulated p53 and p21 expressions. DK115-induced p21 was not observed in HCT116 cells lacking the p53 gene ($p53^{-/-}$), suggesting that DK115 induces p21 expression via p53. These data demonstrate that a novel synthetic DK115 compound may possess antitumor activity through the induction of tumor suppressor p53-mediated G1 cell cycle arrest.

Mitochondrial Potential

J Proteome Res. 2011 May 6;10(5):2389-96. doi: 10.1021/pr101218d. Epub 2011 Mar 28.

Proteomics reveals that redox regulation is disrupted in patients with ethylmalonic encephalopathy.

Palmfeldt J, Vang S, Stenbroen V, Pavlou E, Baycheva M, Buchal G, Monavari AA, Augoustides-Savvopoulou P, Mandel H, Gregersen N.
Research Unit for Molecular Medicine, Aarhus University Hospital, Skejby, Denmark. johan.palmfeldt@ki.au.dk

Abstract

Deficiency of the sulfide metabolizing protein ETHE1 is the cause of ethylmalonic encephalopathy (EE), an inherited and severe metabolic disorder. To study the molecular effects of EE, we performed a proteomics study on mitochondria from cultured patient fibroblast cells. Samples from six patients were analyzed and revealed seven differentially regulated proteins compared with healthy controls. Two proteins involved in pathways of detoxification and oxidative/reductive stress were underrepresented in EE patient samples: mitochondrial superoxide dismutase (SOD2) and aldehyde dehydrogenase X (ALDH1B). Sulfide:quinone oxidoreductase (SQRDL), which takes part in the same sulfide pathway as ETHE1, was also underrepresented in EE patients. The other differentially regulated proteins were apoptosis inducing factor (AIFM1), lactate dehydrogenase (LDHB), chloride intracellular channel (CLIC4) and dimethylarginine dimethylaminohydrolase 1 (DDAH1). These proteins have been reported to be involved in encephalopathy, energy metabolism, ion transport, and nitric oxide regulation, respectively. Interestingly, oxidoreductase activity was overrepresented among the regulated proteins indicating that redox perturbation plays an important role in the molecular mechanism of EE. This observation may explain the wide range of symptoms associated with the disease, and highlights the potency of the novel gaseous mediator sulfide.

Biochem Biophys Res Commun. 2011 May 13;408(3):465-70. doi: 10.1016/j.bbrc.2011.04.053. Epub 2011 Apr 19.

A receptor tyrosine kinase inhibitor, Tyrphostin A9 induces cancer cell death through Drp1 dependent mitochondria fragmentation.

Park SJ, Park YJ, Shin JH, Kim ES, Hwang JJ, Jin DH, Kim JC, Cho DH.
Graduate School of East-West Medical Science, Kyung Hee University, Gyeoggi-Do 446-701, Republic of Korea.

Abstract

Mitochondria dynamics controls not only their morphology but also functions of mitochondria. Therefore, an imbalance of the dynamics eventually leads to mitochondria disruption and cell death. To identify specific regulators of mitochondria dynamics, we screened a bioactive chemical compound library and selected Tyrphostin A9, a tyrosine kinase inhibitor, as a potent inducer of mitochondrial fission. Tyrphostin A9 treatment resulted in the formation of fragmented mitochondria filament. In addition, cellular ATP level was decreased and the mitochondrial membrane potential was collapsed in Tyr A9-treated cells. Suppression of Drp1 activity by siRNA or over-expression of a dominant negative mutant of Drp1 inhibited both mitochondrial fragmentation and cell death induced by Tyrpohotin A9. Moreover, treatment of Tyrphostin A9 also evoked mitochondrial fragmentation in other cells including the neuroblastomas. Taken together, these results suggest that Tyrphostin A9 induces Drp1-mediated mitochondrial fission and apoptotic cell death.

FEBS Lett. 2012 Dec 14;586(24):4303-10. doi: 10.1016/j.febslet.2012.10.035. Epub 2012 Oct 31.

Mitochondrial fragmentation caused by phenanthroline promotes mitophagy.

Park SJ, Shin JH, Kim ES, Jo YK, Kim JH, Hwang JJ, Kim JC, Cho DH.
Graduate School of East-West Medical Science, Kyung Hee University, Gyeoggi-Do, South Korea.

Abstract

Mitochondrial dynamics and mitophagy are thought to be important events for the quality control of mitochondria and mitochondria-associated diseases. To identify novel mitophagy modulators, we developed a cell-based screening system and selected 1,10-phenanthroline (Phen) as a target molecule. Phen treatment highly induced mitochondrial fragmentation and mitochondrial dysfunctions in a Drp1 dependent manner. Phen treatment also increased autophagy. Moreover, prolonged exposure of Phen increased mitochondria clearance through mitophagy. Phen-mediated loss of mitochondrial mass was more reduced in ATG5 deficient cells than in wild type cells. In addition, down-regulation of Drp1 decreased autophagy activation, suggesting that mitochondrial fission is involved in Phen-mediated mitophagy. Thus, our results demonstrate that the disruption of mitochondrial dynamics and mitochondrial dysfunctions provokes mitophagy in Phen-treated cells.

BMB Rep. 2011 Aug;44(8):517-22.

Niclosamide induces mitochondria fragmentation and promotes both apoptotic and autophagic cell death.

Park SJ, Shin JH, Kang H, Hwang JJ, Cho DH.
Graduate School of East-West Medical Science, Kyung Hee University, Yongin, Korea.

Abstract

Mitochondrial dynamics not only involves mitochondrial morphology but also mitochondrial biogenesis, mitochondrial distribution, and cell death. To identify specific regulators to mitochondria dynamics, we screened a chemical library and identified niclosamide as a potent inducer of mitochondria fission. Niclosamide promoted mitochondrial fragmentation but this was blocked by down-regulation of Drp1. Niclosamide treatment resulted in the disruption of mitochondria membrane potential and reduction of ATP levels. Moreover, niclosamide led to apoptotic cell death by caspase-3 activation. Interestingly, niclosamide also increased autophagic activity. Inhibition of autophagy suppressed niclosamide-induced cell death. Therefore, our findings suggest that niclosamide induces mitochondria fragmentation and may contribute to apoptotic and autophagic cell death.

Transfusion. 2013 Jun 24. doi: 10.1111/trf.12307.

A new system for quality control in hematopoietic progenitor cell units before reinfusion in autologous transplant.

Scerpa MC, Rossi C, Daniele N, Lanti A, Adorno G, Picardi A, Arcese W, Amadori S, Isacchi G, Zinno F.

Cryolab Center of Biotechnology and Cryobiology, Immunohematology Section, SIMT, Department of Hematology, Tor Vergata University, Rome, Italy; Rome Transplant Network, Department of Hematology, Tor Vergata University, Rome, Italy.

Abstract

BACKGROUND:

In our Center, the cell viability, the integrity of the bag, and the clonogenic assay were evaluated before the reinfusion of hematopoietic progenitor cells-apheresis (HPC-A). This quality control (QC) should be made 14 days before the reinfusion to the patient to have the result

STUDY DESIGN AND METHODS:

This study was designed to assess the potential of an automatic cell counting system (NucleoCounter NC-3000, ChemoMetec) in our clinical routine as a support of the clonogenic assay and the cytofluorimetric analysis for the QC of the cryopreserved HPC-A. The cell viability

RESULTS:

The evaluation of the total nucleated cells in preapoptosis measured by 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazol-carbocyanine iodide (JC-1) assay showed a negative correlation (r = -0.43) with the total number of colonies (colony-forming unit [CFU]-granulocyte-

CONCLUSION:

The evaluation of cell functionality by the use of the NucleoCounter NC-3000 is in agreement with results from clonogenic assay and can be considered an effective alternative in the routine laboratory.

Quantitative assesment of apoptotic events using image and flow cytometry

Olaf Nielsen[1] , Anna Fossum[2] and Soren Kjaerulff[3]

[1]Department of Biology, University of Copenhagen, [2]BRIC, University of Copenhagen, [3]ChemoMetec A/S

Abstract

Cell death by apoptosis is a complex, tightly regulated process in which a cell orchestrates its own destruction in response to specific internal or external stimuli. Dysregulation of apoptosis can lead to various physical disorders such as cancer and autoimmunity. Information about apoptosis is commonly obtained either by flow cytometry or fluorescence microscopy.Flow cytometry provides quantitative information for thousands of cells, but does not allow for visualization of the cells. In contrast, fluorescence microscopy provides visual information, but does not allow for easy quantitative measurements of large cell populations. Image based cytometry bridges the gap between these two technologies and allows for simultaneous quantitative analysis and visualization of thousands of cells.
We have employed an image based cytometer, NucleoCounter NC-3000, for quantifying different events in the apoptotic process. Compared to flow cytometric analyses (BD LSRII), NC-3000 demonstrated accurate and precise determination of phosphatidylserine translocation, Caspase 3/7 activation and depolarization of the mitochondrial membrane.

Annexin V

Bioorganic & Medicinal Chemistry - Volume 21, Issue 14, 15 July 2013, Pages 4250–4258

Chromenylchalcones showing cytotoxicity on human colon cancer cell lines and in silico docking with aurora kinases

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[b] Division of Bioscience and Biotechnology, BMIC, Konkuk University, Hwayang-Dong 1, Kwangjin-Ku, Seoul 143-701, South Korea

[c] Department of Applied Chemistry, Dongduk Women's University, Seoul 136-714, South Korea

[d] National Institute of Animal Science, Rural Development Administration, Suwon 441-706, South Korea

Abstract

Due to toxicity problems, various plant-derived compounds have been screened to find the chemotherapeutic agents. As anticancer therapeutic agents, chalcones have advantages such as poor interaction with DNA and low risk of mutagenesity. Chromenones show anticancer activities too. Therefore, hybrids of chalcone and chromenone may be potent chemotherapeutic agents. We prepared 16 synthetic chromenylchalcones and applied a clonogenic long-term survival assay method for them on HCT116 human colorectal cancer cell lines. One of chromenylchalcones tested here, chromenylchalcone 11, showed IC50 of 93.1 nM which can be competed with the IC50 values of well-known flavonoids such as catechin gallate and epicatechin gallate. Further biological experiments including cell cycle analysis, apoptosis assay, Western blot analysis, and immunofluorescent microscopy were carried out for this compound. In addition, in vitro kinases binding assay performed to explain its molecular mechanism demonstrated the compound inhibited aurora kinases. The binding modes between chromenylchalcone 11 and aurora kinases were elucidated using in silico docking experiments. These findings could be used for designing cancer therapeutic or preventive plant-derived chromenylchalcone agents.

Quantitative assesment of apoptotic events using image and flow cytometry

Olaf Nielsen[1] , Anna Fossum[2] and Soren Kjaerulff[3]

[1]Department of Biology, University of Copenhagen, [2]BRIC, University of Copenhagen, [3]ChemoMetec A/S

Abstract

Cell death by apoptosis is a complex, tightly regulated process in which a cell orchestrates its own destruction in response to specific internal or external stimuli. Dysregulation of apoptosis can lead to various physical disorders such as cancer and autoimmunity.

Caspase

Quantitative assesment of apoptotic events using image and flow cytometry

Olaf Nielsen[1] , Anna Fossum[2] and Soren Kjaerulff[3]
[1]Department of Biology, University of Copenhagen, [2]BRIC, University of Copenhagen, [3]ChemoMetec A/S

Abstract
Cell death by apoptosis is a complex, tightly regulated process in which a cell orchestrates its own destruction in response to specific internal or external stimuli. Dysregulation of apoptosis can lead to various physical disorders such as cancer and autoimmunity.

DNA fragmentation

Oncogene. 2013 Mar 21;32(12):1601-8. doi: 10.1038/onc.2012.175. Epub 2012 May 14.

Expression of Id proteins is regulated by the Bcl-3 proto-oncogene in prostate cancer.

Ahlqvist K, Saamarthy K, Syed Khaja AS, Bjartell A, Massoumi R.
Center for Molecular Tumor Pathology, Department of Laboratory Medicine, Lund University, Skåne University Hospital, Malmö, Sweden.

Abstract
B-cell leukemia 3 (Bcl-3) is a member of the inhibitor of κB family, which regulates a wide range of biological processes by functioning as a transcriptional activator or as a repressor of target genes. As high levels of Bcl-3 expression and activation have been detected in different types of human cancer, Bcl-3 has been labeled a proto-oncogene. Our study uncovered a markedly upregulated Bcl-3 expression in human prostate cancer (PCa), where inflammatory cell infiltration was observed. Elevated Bcl-3 expression in PCa was dependent on the proinflammatory cytokine interleukin-6-mediated STAT3 activation. Microarray analyses, using Bcl-3 knockdown in PCa cells, identified the inhibitor of DNA-binding (Id) family of helix-loop-helix proteins as potential Bcl-3-regulated genes. Bcl-3 knockdown reduced the abundance of Id-1 and Id-2 proteins and boosted PCa cells to be more receptive to undergoing apoptosis following treatment with anticancer drug. Our data imply that inactivation of Bcl-3 may lead to sensitization of cancer cells to chemotherapeutic drug-induced apoptosis, thus suggesting a potential therapeutic strategy in PCa treatment.

Int J Mol Sci. 2012;13(4):4351-66. doi: 10.3390/ijms13044351. Epub 2012 Apr 5.

Ultraviolet C Irradiation Induces Different Expression of Cyclooxygenase 2 in NIH 3T3 Cells and A431 Cells: The Roles of COX-2 Are Different in Various Cell Lines.

Tai MH, Weng CH, Mon DP, Hu CY, Wu MH.
Institute of Biomedical Sciences, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan; E-Mail: minghongtai@gmail.com.

Abstract
Ultraviolet C (UVC) is a DNA damage inducer, and 20 J/m(2) of UVC irradiation caused cell growth inhibition and induced cell death after exposure for 24-36 h. The growth of NIH 3T3 cells was significantly suppressed at 24 h after UVC irradiation whereas the proliferation of A431 cells was inhibited until 36 h after UVC irradiation. UVC irradiation increased COX-2 expression and such up-regulation reached a maximum during 3-6 h in NIH 3T3 cells. In contrast, UVC-induced COX-2 reached a maximum after 24-36 h in A431 cells. Measuring prostaglandin E2 (PGE2) level showed a biphasic profile that PGE2 release was rapidly elevated in 1-12 h after UVC irradiation and increased again at 24 h in both cell lines. Treatment with the selective COX-2 inhibitor, SC-791, during maximum expression of COX-2 induction, attenuated the UVC induced-growth inhibition in NIH 3T3 cells. In contrast, SC-791 treatment after UVC irradiation enhanced death of A431 cells. These data showed that the patterns of UVC-induced PGE2 secretion from NIH 3T3 cells and A431 cells were similar despite the differential profile in UVC-induced COX-2 up-regulation. Besides, COX-2 might play different roles in cellular response to UVC irradiation in various cell lines.

OJApo> Vol.2 No.2, April 2013

Novel Nitrobenzazolo[3,2-a]quinolinium Salts Induce Cell Death through a Mechanism Involving DNA Damage, Cell Cycle Changes, and Mitochondrial Permeabilization

Christian Vélez, Osvaldo Cox, Carlos A. Rosado-Berrios, Dennise Molina, Luz Arroyo, Sujey Carro, Anton Filikov, Vineet Kumar, Sanjay V. Malhotra, Marisol Cordero, Beatriz Zayas

Abstract
This study reports the capacity of three nitro substituted benzazolo[3,2-a]quinolinium salts NBQs: NBQ 95 (NSC-763304), NBQ 38 (NSC 763305), and NBQ 97 (NSC-763306) as potential antitumor agents. NBQ's are unnatural alkaloids possessing a positive charge that could facilitate interaction with cell organelles. The anticancer activities of these compounds were evaluated through the National Cancer Institute (NCI) 60 cell line screening which represents diverse histologies. The screening was performed at 10 μM on all cell lines. Results from the NCI screening indicated cytotoxicity activity on six cell lines. In order to explore a possible mechanism of action, a detailed biological activity study of NBQ 95 and NBQ 38 was performed on A431 human epidermoid carcinoma cells to determine an apoptotic pathway involving, cell cycle changes, DNA fragmentation, mutations, mitochondrial membrane permeabilization and caspases activation. DNA fragmentation, cell cycle effects, mutagenesis, mitochondrial permeabilization and activation of caspases were determined by fluorimetry and differential imaging. Our data showed that A431 growth was inhibited with an average IC50 of 30 mM. In terms of the mechanism, these compounds interacted with DNA causing fragmentation and cell cycle arrest at sub G0/G1 stage. Mutagenesis was higher for NBQ 38 and moderate for NBQ 95 Mitochondrial permeabilization was observed with NBQ 38 and slightly for NBQ 95. Both compounds caused activation of Caspases 3 and 7 suggesting an apoptotic cell death pathway through an intrinsic mechanism. This study reports evidence of the toxicity of these novel compounds with overlapping structural and mechanistic similarities to ellipticine, a known anti-tumor compound.

Pannem, R. R., et al Carcinogenesis. 2013 Nov 8

CYLD controls c-MYC expression through the JNK-dependent signaling pathway in hepatocellular carcinoma

Pannem RR, Dorn C, Ahlqvist K, Bosserhoff AK, Hellerbrand C, Massoumi R.

Abstract

Posttranslational modification of different proteins via direct ubiquitin attachment is vital for mediating various cellular processes. Cylindromatosis (CYLD), a deubiquitination enzyme, is able to cleave the polyubiquitin chains from the substrate and to regulate different signaling pathways. Loss, or reduced expression, of CYLD is observed in different types of human cancer, such as hepatocellular carcinoma (HCC). However, the molecular mechanism by which CYLD affects cancerogenesis has to date not been unveiled. The aim of the present study was to examine how CYLD regulates cellular functions and signaling pathways during hepatocancerogenesis. We found that mice lacking CYLD were highly susceptible to chemically induced liver cancer. The mechanism behind proved to be an elevated proliferation rate of hepatocytes, owing to sustained c-Jun N-terminal kinase 1 (JNK1)-mediated signaling via ubiquitination of TNF receptor-associated factor 2 and expression of c-MYC. Overexpression of wild-type CYLD in HCC cell lines prevented cell proliferation, without affecting apoptosis, adhesion and migration. A combined immunohistochemical and tissue microarray analysis of 81 human HCC tissues revealed that CYLD expression is negatively correlated with expression of proliferation markers Ki-67 and c-MYC. To conclude, we found that downregulation of CYLD induces tumor cell proliferation, consequently contributing to the aggressive growth of HCC. Our findings suggest that CYLD holds potential to serve as a marker for HCC progression, and its link to c-MYC via JNK1 may provide the foundation for new therapeutic strategies for HCC patients.

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Evidence-Based Complementary and Alternative MedicineVolume 2013 (2013), Article ID 632121, 8 pages

Resveratrol Inhibits Alpha-Melanocyte-Stimulating Hormone Signaling, Viability, and Invasiveness in Melanoma Cells

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[2]Institute of Traditional Medicine, National Yang Ming University, Taipei 112, Taiwan

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[4]Department of Biochemical Science and Technology, National Chiayi University, 300 University Road, Chiayi 600, Taiwan

Abstract

Melanoma is a malignancy with high potential to invasion and treatment resistance. The α-melanocyte-stimulating hormone (α-MSH) signal transduction involving Wnt/β-catenin, c-Kit, and microphthalmia-associated transcription factor (MITF), a known pathway to produce melanin, has been demonstrated as one of cancer stem cell characteristics. This study was aimed to examine the effect of resveratrol, an abundant ingredient of grape and medicinal plants, on α-MSH signaling, viability, and invasiveness in melanoma cells. By α-MSH treatment, the melanin production in B16 melanoma cells was augmented as a validation for activation of α-MSH signaling. The upregulated expression of α-MSH signaling-related molecules β-catenin, c-Kit, and MITF was suppressed by resveratrol and/or STI571 treatment. Nuclear translocation of MITF, a hallmark of α-MSH signaling activation, was inhibited by combined treatment of resveratrol and STI571. At effective concentration, resveratrol and/or STI571 inhibited cell viability and α-MSH-activated matrix metalloproteinase- (MMP-)9 expression and invasion capacity of B16 melanoma cells. In conclusion, resveratrol enhances STI571 effect on suppressing the α-MSH signaling, viability, and invasiveness in melanoma cells. It implicates that resveratrol may have potential to modulate the cancer stem cell characteristics of melanoma.

Human molecular genetics Volume 2013 (2013), Article ID 632121, 8 pages

Secondary coenzyme Q10 deficiency and oxidative stress in cultured fibroblasts from patients with riboflavin responsive multiple Acyl-CoA dehydrogenation deficiency

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[5]Department of Biomedicine, Aarhus University, Denmark

Abstract

Coenzyme Q10 (CoQ10) is essential for the energy production of the cells and as an electron transporter in the mitochondrial respiratory chain. CoQ10 links the mitochondrial fatty acid β-oxidation to the respiratory chain by accepting electrons from electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO). Recently, it was shown that a group of patients with the riboflavin responsive form of multiple acyl-CoA dehydrogenation deficiency (RR-MADD) carrying inherited amino acid variations in ETF-QO also had secondary CoQ10 deficiency with beneficial effects of CoQ10 treatment, thus adding RR-MADD to an increasing number of diseases involving secondary CoQ10 deficiency. In this study, we show that moderately decreased CoQ10 levels in fibroblasts from six unrelated RR-MADD patients were associated with increased levels of mitochondrial reactive oxygen species (ROS). Treatment with CoQ10, but not with riboflavin, could normalize the CoQ10 level and decrease the level of ROS in the patient cells. Additionally, riboflavin depleted control fibroblasts showed moderate CoQ10 deficiency, but not increased mitochondrial ROS, indicating that variant ETF-QO proteins and not CoQ10 deficiency are the causes of mitochondrial ROS production in the patient cells. Accordingly, the corresponding variant Rhodobacter sphaeroides ETF-QO proteins, when overexpressed in vitro, bind a CoQ10 pseudosubstrate, Q10Br, less tightly than the wild-type ETF-QO protein, suggesting that molecular oxygen can get access to the electrons in the misfolded ETF-QO protein, thereby generating superoxide and oxidative stress, which can be reversed by CoQ10 treatment.

Cancer Res. 2013 Mar 15;73(6):1934-45. doi: 10.1158/0008-5472.CAN-12-3682. Epub 2013 Mar 6.

Alkaline phosphatase ALPPL-2 is a novel pancreatic carcinoma-associated protein.

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Department of Medical Biotechnology, Dongguk University, Seoul, Korea.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a very low median survival rate. The lack of early sensitive diagnostic markers is one of the main causes of PDAC-associated lethality. Therefore, to identify novel pancreatic cancer biomarkers that can facilitate early diagnosis and also help in the development of effective therapeutics, we developed RNA aptamers targeting pancreatic cancer by Cell-systematic evolution of ligands by exponential enrichment (SELEX) approach. Using a selection strategy that could generate aptamers for 2 pancreatic cancer cell lines in one selection scheme, we identified an aptamer SQ-2 that could recognize pancreatic cancer cells with high specificity. Next, by applying 2 alternative approaches: (i) aptamer-based target pull-down and (ii) genome-wide microarray-based identification of differentially expressed mRNAs in aptamer-positive and -negative cells, we identified alkaline phosphatase placental-like 2 (ALPPL-2), an oncofetal protein, as the target of SQ-2. ALPPL-2 was found to be ectopically expressed in many pancreatic cancer cell lines at both mRNA and protein levels. RNA interference-mediated ALPPL-2 knockdown identified novel tumor-associated functions of this protein in pancreatic cancer cell growth and invasion. In addition, the aptamer-mediated identification of ALPPL-2 on the cell surface and cell secretions of pancreatic cancer cells supports its potential use in the serum- and membrane-based diagnosis of PDAC.

Mol Cells. 2013 Apr;35(4):320-6. doi: 10.1007/s10059-013-2316-7. Epub 2013 Apr 4.

RNA interference-mediated simultaneous silencing of four genes using cross-shaped RNA.

Lee TY, Chang CI, Lee D, Hong SW, Shin C, Li CJ, Kim S, Haussecker D, Lee DK.

Global Research Laboratory for RNAi Medicine, Department of Chemistry, Sungkyunkwan University, Suwon 440-746, Korea.

Abstract

The structural flexibility of RNA interference (RNAi)-triggering nucleic acids suggests that the design of unconventional RNAi trigger structures with novel features is possible. Here, we report a cross-shaped RNA duplex structure, termed quadruple interfering RNA (qiRNA), with multiple target gene silencing activity. qiRNA triggers the simultaneous down-regulation of four cellular target genes via an RNAi mechanism. In addition, qiRNA shows enhanced intracellular delivery and target gene silencing over conventional siRNA when complexed with jetPEI, a linear polyethyleneimine (PEI). We also show that the long antisense strand of qiRNA is incorporated intact into an RNA-induced silencing complex (RISC). This novel RNA scaffold further expands the repertoire of RNAi-triggering molecular structures and could be used in the development of therapeutics for various diseases including viral infections and cancer.

DNA Repair (Amst). 2012 Nov 1;11(11):892-905. doi: 10.1016/j.dnarep.2012.08.005. Epub 2012 Sep 24.

RNF8 and RNF168 but not HERC2 are required for DNA damage-induced ubiquitylation in chicken DT40 cells.

Oestergaard VH, Pentzold C, Pedersen RT, Iosif S, Alpi A, Bekker-Jensen S, Mailand N, Lisby M.

Department of Biology, University of Copenhagen, DK-2200, Copenhagen N, Denmark.

Abstract

The ubiquitylation cascade plays an important role in the recruitment of repair factors at DNA double-strand breaks. The involvement of a growing number of ubiquitin E3 ligases adds to the complexity of the DNA damage-induced ubiquitin signaling. Here we use the genetically tractable avian cell line DT40 to investigate the role of HERC2, RNF8 and RNF168 in the DNA damage-induced ubiquitylation pathway. We show that formation of ubiquitin foci as well as cell survival after DNA damage depends on both RNF8 and RNF168. However, we find that RNF8 and RNF168 knockout cell lines respond differently to treatment with camptothecin indicating that they do not function in a strictly linear manner. Surprisingly, we show that HERC2 is required neither for survival nor for ubiquitin foci formation after DNA damage in DT40. Moreover, the E3 ubiquitin ligase activity of HERC2 is not redundant to that of RNF8 or RNF168.

Mol Cancer Ther. 2013 Aug 6.

Sunitinib and SU11652 inhibit acid sphingomyelinase, destabilize lysosomes and inhibit multidrug resistance

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1Cell Death and Metabolism, Danish Cancer Society Research Center.

Abstract

Defective apoptosis signaling and multidrug resistance are major barriers for successful cancer treatment. To identify drugs capable of targeting treatment resistant cancer cells, we screened small molecule kinase inhibitor libraries for compounds that decrease the viability of apoptosis-resistant human MCF7-Bcl-2 breast cancer cells. SU11652, a multi-targeting receptor tyrosine kinase inhibitor, emerged as the most potent compound in the screen. In addition to MCF7-Bcl-2 cells, it effectively killed HeLa cervix carcinoma, U-2-OS osteosarcoma, Du145 prostate carcinoma and WEHI-S fibrosarcoma cells at low micromolar concentration. SU11652 accumulated rapidly in lysosomes and disturbed their pH regulation and ultrastructure eventually leading to the leakage of lysosomal proteases into the cytosol. Lysosomal destabilization was preceded by an early inhibition of acid sphingomyelinase (ASM), a lysosomal lipase that promotes lysosomal membrane stability. Accordingly, heat shock protein 70, which supports cancer cell survival by increasing lysosomal ASM activity, conferred partial protection against SU11652-induced cytotoxicity. Remarkably, SU11652 killed multidrug resistant Du145 prostate cancer cells as effectively as the drug-sensitive parental cells, and subtoxic concentrations of SU11652 effectively inhibited multidrug resistant phenotype in Du145 prostate cancer cells. Notably, sunitinib, a structurally almost identical and widely used anti-angiogenic cancer drug, exhibited similar lysosome-dependent cytotoxic activity, albeit with significantly lower efficacy. The significantly stronger lysosome-targeting activity of SU11652 suggests that it may display better efficacy in cancer treatment than sunitinib encouraging further evaluation of its anti-cancer activity in vivo. Furthermore, our data provide a rationale for novel approaches to target drug resistant cancers by combining classic chemotherapy with sunitinib or SU11652.
