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Pathogenetic-based toxic-effects of cadmium chloride in Wistar rats

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Abstract

The current study investigated the toxic effects of cadmium chloride ($CdCl_2$) on the livers of Wistar rats using both histopathological and genetic features as indicators. Here, 12 rats were randomly categorized into 3 groups (4 animals/group). One group received 8mg/kg B.W IP of $CdCl_2$ daily for 30 days (8G). Another group was given 4mg/kg B.W IP of $CdCl_2$ daily for 30 days (4G). The last group was subjected to distilled water only, a control group, (CG). After the 30-day experiment, all animals were sacrificed, and samples from liver tissues and bile ducts were collected for p53 gene expression (P53GE), using real time PCR (RT-PCR), and histopathological tests. The histopathological findings revealed lesions such as hepatic degeneration, vacuolation of the hepatocytes, hyperplasia in the bile ducts, and dilatation of sinusoids. For the P53GE, significant increases were shown in the 4G, 6.672, greater than those in the 8G, 2.838, when compared with the CG, 0.971. In conclusion, $CdCl_2$ induces detrimental toxic effects shown via the histopathological changes in the liver and the bile duct tissues. The P53 increased expression in the 4G might indicate suppressing action against a possible activation of $CdCl_2$-induced cancer in those tissues.

Keywords: Cadmium chloride, histopathological, P53, Wistar rats.

INTRODUCTION

Cadmium (Cd) is a well-known heavy metal for its toxicity, and it is present in the environment in different forms. Industries involving cadmium are huge in numbers such as battery, plastic, fertilizer, cigarette, mobile chargers, and paints productions. No good strategic use of this toxic element leads to contamination of soil and water in the affected areas. Direct contact with Cd may induce toxicity to all body organs and tissues. People and animals are affected by such toxicity via direct or indirect contact with Cd such as via inhalation or skin contact (1,2).

Many studies showed that $CdCl_2$ causes excessive production of reactive oxygen species (ROS) and leads to damages in the affected tissues of humans or animals (3,4). Another scenario of $CdCl_2$ toxicity is that DNA damage induced via lipid peroxidation (5). For certain pathways, $CdCl_2$ settles down in the kidney and liver tissues, and it results in changes in the histopathological and the gene expression levels of tumor-suppressor genes such as p53 (6,7). P53 gene codes for a protein called TP53 that has some important roles in cell cycle, apoptosis and growth arrest, suppressing cancer cell, and repairing DNA damages. DNA-related damages induce more production of p53 protein (8–10). Many reports showed, using microtome, histopathological changes induced in acute and chronic toxicities via the exposure to $CdCl_2$ (11–13).

The main purpose of the current study was to understand the effects of $CdCl_2$ on some tissues such as those belong to livers and bile ducts in Wistar rats. The study also was focused on the effects of $CdCl_2$ on the expression of the p53 gene via the estimation of gene expression by a RT-PCR technique.

MATERIALS AND METHODS

Animals and Experimental design

Here, 12 Wistar male and female rats, 200-220gm, were purchased from the animal housing in the College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq. The animals were randomly categorized into 3 groups (4 animals/group). One group received 8mg/kg B.W IP of $CdCl_2$ daily for 30 days (8G). Another group was given 4mg/kg B.W IP of $CdCl_2$ daily for 30 days (4G). The last group was subjected to 0.2ml of distilled water only, a control group, (CG). The $CdCl_2$ was obtained from the Central Laboratories of the same university. The rats were housed during the experiment period, 30 days, in suitable plastic cages placed in well-ventilated rooms, 25±2 ^{0}C in the College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq, and 12/12hrs of light/dark cycle was provided. Food as pellets was given plus water was provided ad libitum. After the 30-day experiment, all animals were sacrificed, and samples from liver tissues and bile ducts were collected. Samples were placed in 1.5ml tubes preloaded with DEPC water and snapped frozen in liquid nitrogen to be stored in a -20 ^{0}C freezer in the same college. P53 gene expression (P53GE), using real time PCR (RT-PCR), and histopathological tests were performed in the mentioned college. For the histopathological examination, 2cm³ of liver tissues was obtained, fixed in a 10%-formaldehyde solution, and processed using a histokinette (SLEE medical, Germany). The technique used were followed from (14).

Total RNA extraction

The kit used for the total RNA extraction was Accuzol® reagent kit (Bioneer, Korea). The instructions accompanied the kit were followed. To initiate the process, 200mg of liver or bile duct tissues was used. The resulted extract of RNA was kept frozen in -20 ^{0}C. DNase I, DNase I Kit (Promega, USA), was used, to get rid of any remaining of genomic DNA, according to the kit instructions.

Reverse-transcription and RT-PCR

To reverse-transcribe mRNA into cDNA, AccuPower® RocktScript RT PreMix (Bioneer, Korea). The kit instructions were followed to perform the process. The temperatures used for the process were 50 ^{0}C for 1hr to synthesize cDNA (RT step), and 95 ^{0}C for 5min to inactivate heat. RT-PCR was used to quantitate p53GE using the 2-ΔΔCT Livak method (15). The PCR system used was from (BioRad, USA) using SYBER Green dye qPCR master mix. The primers for the p53 gene, NM_030989.3, were F: ATCCTATCCGGTCAGTTGTTGG and R: AATGCAGACAGGCTTTGCAG to amplify a 143bp region. The β-actin primers, NM_031144.3, were F: CTAGGCACCAGGGTGTGATG and R: GTCAGGATGCCTCTCTTGCTC to amplify an 85bp region. The master mix solutions were generated using AccuPowerTM 2XGreen Star qPCR master mix kit (Bioneer, Korea). The instructions for the kit were followed. The condition temperatures used were 50 ^{0}C for 1hr to begin the denaturation for 1 cycle, 45 cycle (95 ^{0}C for 20s to do the main denaturation and 60 ^{0}C for 30s to do the annealing/extension/detection), and 60-95 ^{0}C for 0.5s for 1 cycle.

RESULTS

Histopathological changes

The histopathological findings revealed lesions such as hepatic degeneration, vacuolation of the hepatocytes, infiltrations of inflammatory cells, hyperplasia in the bile ducts, dilatation of sinusoids, mild congestion of the central vein, and mild dilatation of sinusoid, figure 1, 2, 3, 4, 5, and 6.

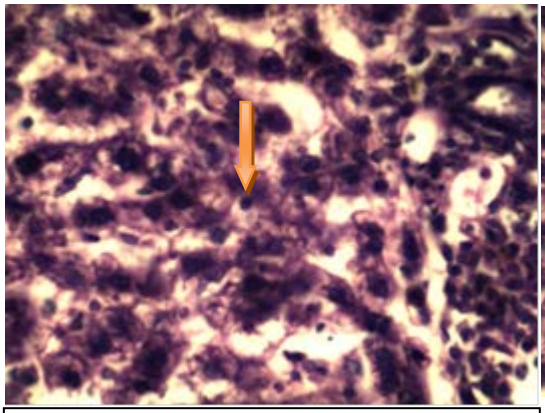

Figure 1: Histological section of liver of rat treated with $CdCl_2$ (8G). It shows fatty-degenerated hepatocyte characterized by signate like shape, peripheral nuclei, and hepatocyte vaccoulation (40XH&E).

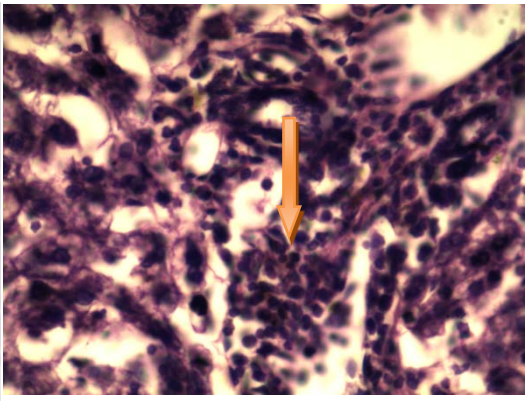

Figure 2: Histological section of liver of rat treated with $CdCl_2$ (8G) shows infiltration of inflammatory cells in the hepatic tissue (40XH&E).

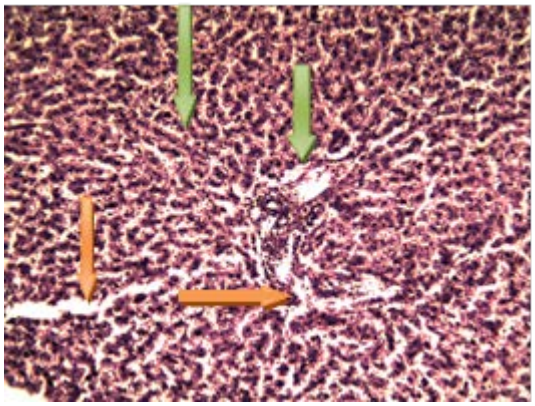

Figure 3: Histological section of liver of rat treated with $CdCl_2$ (8G) shows hyperplasia of bile duct (green arrow) with dilation of the sinusoid (10XH&E).

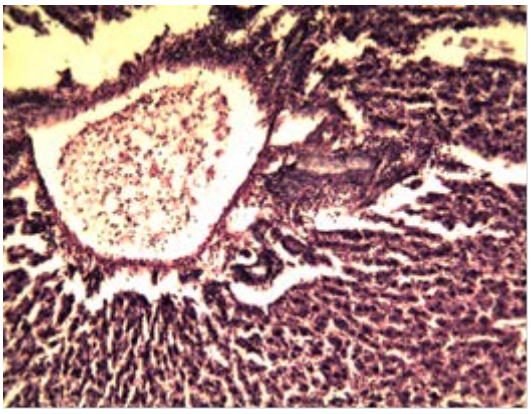

Figure 4: Histological section of liver of rat treated with $CdCl_2$ (8G) shows mild congestion of the central vein (green arrow) and mild dilatation of sinusoid (10XH&E).

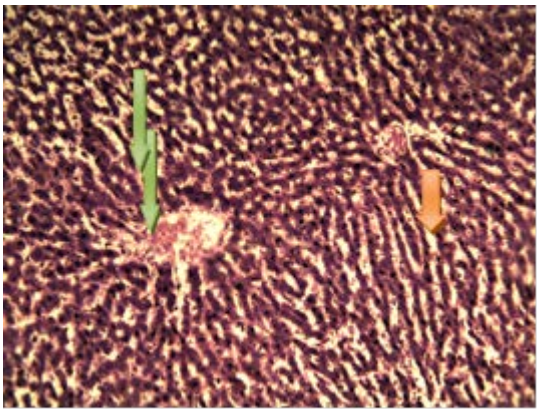

Figure 5: Histological section of liver of rat treated with $CdCl_2$ (4G) shows mild congestion of the central vein (green arrow) and mild dilatation of sinusoid (10XH&E).

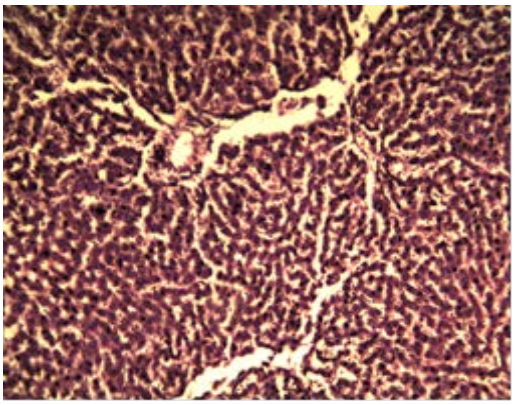

Figure 6: Histological section of liver of rat treated with $CdCl_2$ (4G) shows cytoarchitecture of hepatic lobules consisting of central veins and radially arranged hepatocytes (10XH&E).

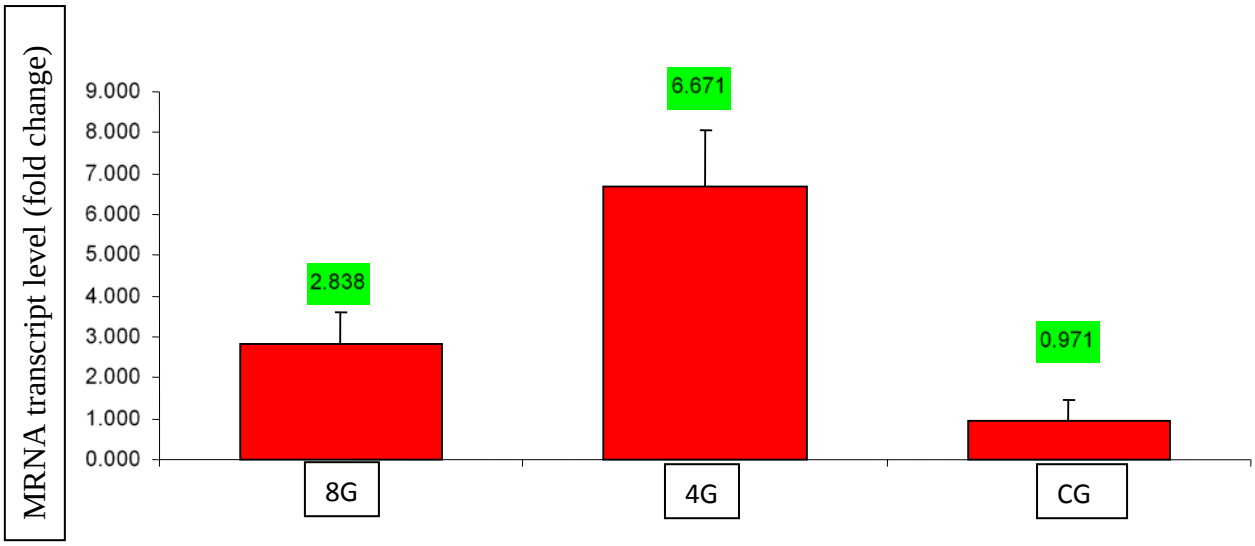

Figure 7: Relative mRNA level of p53 in the liver of rats injected with $CdCl_2$ (4G, 4mg/kg B.W) and (8G, 8mg/kg B.W) for 30th days was determined using RT-PCR.

P53 gene expression

For the P53GE, significant increases were shown in the 4G, 6.672, greater than those in the 8G, 2.838, when compared with the CG, 0.971, figure 7.

DISCUSSION

The current study showed severe histopathological changes in the livers of the treated rats with $CdCl_2$ as the liver is the main metabolizing site of this agent. These results agree with (16,17) who found vacuolar- and granular-based degeneration, dilatation in the sinusoids and the portal triad in the liver histopathological pictures of rat exposed to Cd. Some researchers found that Cd induced dilatation of sinusoids, mild inflammation, and micro and macrosteatosis (12). The present findings the current study also match up with (18) who showed spread hepatic steatosis, diffuse necrobiosis, and infiltrations of inflammatory cells. For the genetic-based changes, (19) indicated that exposure to Cd at 50mg for 12 weeks induced compact of chromatin. Moreover, liver cell cytoplasm became light, and trabecular structure was blurred, foamy and vacuolated. The cells were enlarged with small nucleoli. However, mononuclear cells accumulation in the sinusoids pycnotic with condensed chromatin and accumulation of Kupffer cells in the walls of sinusoids disagreed with the current study results. The presence of inflammatory cells infiltrations indicates severe histopathological reactions generated against Cd (20,21).

Significant increases in the p53 gene expression was higher in 4G than that in 8G. Such increases are linked to the lower dose, 4mg, in the 4G that may have not induced heavily damages in the genetic materials, DNA, and p53 was still activated. However, the higher dose,8mg, in 8G might have been enough to destroy all the DNA and no more p53 was induced (22). The P53GE results agree with (23,24) who found genetic response via p53 expression that was linked to the dose of Cd taken. P53-dependent pathway induces apoptosis via the exposure to Cd (24). When p53 is inactivated or lost, this might increase the occurrence rate of malignant tumors (25). The loss of p53 may induce failure in the cell arrest during the G1 phase of the cell cycle when exposing to DNA damage (26). So, this could be a protection mechanism induced in the cells affected by Cd. In conclusion, $CdCl_2$ induces detrimental toxic effects shown via the histopathological changes in the liver and the bile duct tissues. The P53 increased expression in the 4G might indicate suppressing action against a possible activation of $CdCl_2$-induced cancer in those tissues. This heavy metal must be controlled to keep it out the acceptable concentrations in the environment.

REFERENCES

1. Dzobo K, Naik YS. Effect of selenium on cadmium-induced oxidative stress and esterase activity in rat organs. S Afr J Sci [Internet]. 2013, May 20 [cited 2018 Sep 11];109(5/6):1–8. Available from: http://sajs.co.za/article/view/3884
2. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med [Internet]. 1995 Feb [cited 2018, Sep 11];18(2):321–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7744317
3. Thijssen S, Maringwa J, Faes C, Lambrichts I, Van Kerkhove E. Chronic exposure of mice to environmentally relevant, low doses of cadmium leads to early renal damage, not predicted by blood or urine cadmium levels. Toxicology [Internet]. 2007 Jan [cited 2018, Sep 12];229(1–2):145–56. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0300483X06006159
4. THOMPSON J, BANNIGAN J. Cadmium: Toxic effects on the reproductive system and the embryo. Reprod Toxicol [Internet]. 2008 Apr [cited 2018, Sep 12];25(3):304–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18367374
5. Sugiyama M. Role of physiological antioxidants in chromium(VI)-induced cellular injury. Free Radic Biol Med [Internet]. 1992, [cited 2018 Sep 12];12(5):397–407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1592274
6. Stohs SJ, Bagchi D, Hassoun E, Bagchi M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol [Internet]. 2000 [cited 2018, Sep 12];19(3):201–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10983887
7. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology [Internet]. 2003 Nov 5 [cited 2018, Sep 12];192(2–3):95–117. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14580780
8. Zhan Q, Wang L, Song Y, Ou Y, Jiang J, Fan J, et al. Esophageal Carcinoma. Recent Adv Cancer Res Ther [Internet]. 2012, Jan 1 [cited 2018 Sep 12];493–534. Available from: https://www.sciencedirect.com/science/article/pii/B9780123978332000182
9. Bhargava R, Esposito NN. Immunohistology of the Breast. Diagnostic Immunohistochem [Internet]. 2011, Jan 1 [cited 2018 Sep 12];763–819. Available from: https://www-sciencedirect-com.ezproxy.snhu.edu/science/article/pii/B9781416057666000236
10. Tang Y, Long J, Liu J. Hyperglycemia-Associated Oxidative Stress Induces Autophagy: Involvement of the ROS-ERK/JNK-p53 Pathway. Autophagy Cancer, Other Pathol Inflammation, Immunity,

Infect Aging [Internet]. 2014, Jan 1 [cited 2018 Sep 12]; 105–15. Available from: https://www.sciencedirect.com/science/article/pii/B978012405530800008X?via%3Dihub

11. Beširovi H, Ali AS, Prašovi S, Drommer W. Histopathological Effects of Chronic Exposure to Cadmium and Zinc on Kidneys and Gills of Brown Trout [Internet]. 2010 [cited 2018, Sep 12]. Available from: https://www.semanticscholar.org/paper/Histopathological-Effects-of-Chronic-Exposure-to-on-Beširovi-Ali/4f91b7894b72c1109044dbac71f72e1f4ccc9cab
12. Dardouri K, Haouem S, Gharbi I, Sriha B, Haouas Z, Hani A El, et al. Combined Effects of Cd and Hg on Liver and Kidney Histology and Function in Wistar Rats. J Agric Chem Environ [Internet]. 2016, Oct 19 [cited 2018 Sep 12];05(04):159–69. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/jacen.2016.54017
13. Lu H, Yuan G, Yin Z, Dai S, Jia R, Xu J, et al. Effects of subchronic exposure to lead acetate and cadmium chloride on rat's bone: Ca and Pi contents, bone density, and histopathological evaluation. Int J Clin Exp Pathol [Internet]. 2014, [cited 2018 Sep 12];7(2):640–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24551284
14. Kuerbitz SJ, Plunkett BS, Walsh W V, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci U S A [Internet]. 1992, Aug 15 [cited 2018 Sep 12]; 89(16):7491–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1323840
15. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods [Internet]. 2001 Dec [cited 2018, Sep 12]; 25(4):402–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11846609
16. Koyu A, Gokcimen A, Ozguner F, Bayram DS, Kocak A. Evaluation of the effects of cadmium on rat liver. Mol Cell Biochem [Internet]. 2006, May 12 [cited 2018 Sep 12];284(1–2):81–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16424996
17. Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Mol Toxicol [Internet]. 2000, [cited 2018 Sep 12];14(2):110–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10630425
18. Coman M, Stancu A. Hepatic and renal histopathological aspects in rats intoxicated with cadmium chloride treated preventively and curatively with water aloe extract. 2008; xli:721–6.
19. Brzóska MM, Moniuszko-Jakoniuk J, Piłat-Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol [Internet]. 2003, [cited 2018 Sep 12];38(1):2–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12554600
20. Klaassen CD, Liu J. Role of metallothionein in cadmium-induced hepatotoxicity and nephrotoxicity. Drug Metab Rev [Internet]. [cited 2018, Sep 12];29(1–2):79–102. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9187512
21. Nordberg M, Nordberg GF. On the role of metallothionein in cadmium induced renal toxicity. Experientia Suppl [Internet]. 1987, [cited 2018 Sep 12];52:669–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2959560
22. Urani C, Melchioretto P, Fabbri M, Bowe G, Maserati E, Gribaldo L. Cadmium Impairs p53 Activity in HepG2 Cells. ISRN Toxicol [Internet]. 2014, Mar 13 [cited 2018 Sep 12];2014:976428. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25101185
23. Xu G, Zhou G, Jin T, Zhou T, Hammarström S, Bergh A, et al. Apoptosis and p53 gene expression in male reproductive tissues of cadmium exposed rats. Biometals [Internet]. 1999, Jun [cited 2018 Sep 12];12(2):131–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10406082
24. Westin P, Brändström A, Damber JE, Bergh A. Castration plus oestrogen treatment induces but castration alone suppresses epithelial cell apoptosis in an androgen-sensitive rat prostatic adenocarcinoma. Br J Cancer [Internet]. 1995, Jul [cited 2018 Sep 12];72(1):140–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7599043
25. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell [Internet]. 1992, Sep 18 [cited 2018 Sep 12];70(6):923–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1356076
26. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell [Internet]. 1992, Sep 18 [cited 2018 Sep 12];70(6):937–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1525830