Estimation of HSP70 in SiHa Cell Lines During Chemoradiation and Radiation

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Abstract. Cervical cancer remains a significant health problem worldwide, despite the advances in screening, diagnosis, treatment, and prognosis. Heat shock protein 70 (HSP70) is an anti-apoptotic protein that inhibits the cancer cell death by interfering with apoptotic pathway activation. In chemoradiation, the production of the Reactive Oxygen Species damages the organelles of the cancer cells. This urges the dying cells to produce HSP70 which inhibits the extrinsic and intrinsic apoptotic pathways helping in the prognosis of cancer. In the present study before approaching human subjects, the hypothesis is tested on SiHa cervical squamous cell carcinoma cell line. The paper aims to estimate the anti-apoptotic HSP70 protein in the media collected from the cultured SiHa cervical squamous cell carcinoma cell line treated with cisplatin, radiation and chemoradation. The Hsp70 is estimated using ELISA method and the HSPA1A gene expression is estimated using qRT-PCR. From the radiation and chemoradiation results, the investigators noticed that cancer treatments induce cellular damage and initiate a cytoprotective mechanism in cells as a response. Higher levels of HSP70 are found in SiHa cells treated with cisplatin-combined radiation when compared to cisplatin or radiation alone. From the findings, the investigators conclude that the higher expression levels of HSP70 in chemoradiated SiHa cells showed that cytoprotective anti-apoptotic HSP70 protein is released as a response to chemoradiation.

Keywords: Heat shock protein 70 · cancer cell lines · Cervical cancer · chemotherapy · radiation

1 Introduction

According to the latest Globocan report 2021, in India 1,23,907 women are diagnosed with cervical cancer and 77,348 died from the disease in 2020 [1]. HPV information center 2020 stated that, the second most prevalent cancer in India is cervical cancer. It is the primary cause associated to cancer deaths in women in middle and low-income countries [2]. In clinical studies, before animal / human studies the hypothesis is tested on cell lines. Cell culture is selected as the primary study to understand molecular, and cellular biology (e.g., the interaction between cells, responses to drugs/treatments, etc.).

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Cell lines are immensely helpful for cancer pharmacogenomic studies to predict clinical response, and drug testing. These studies help to uncover novel mechanisms underlying variations in drug response. The protein up/down regulations or cellular cytotoxicity can also be easily verified [3]. In the present study, the investigators adopted the cell culture method for culturing SiHa cells. The Heat Shock Proteins (HSPs) namely HSP60, HSP70, HSP90 are a group of proteins that belong to chaperone family. Interestingly, HSP70 is an important stress-inducible and anti-apoptotic protein [4]. HSP70 is essential for cell survival in stressful situations. HSP70 functions as a molecular chaperone, helping misfolded proteins accumulated by stress to fold correctly and avoid cell death [5]. Stabilization of oncogenic proteins, suppression of apoptosis, inhibition of replicative senescence, tumor angiogenesis, induction, invasion and stimulation of metastasis are some of its roles in carcinogenesis [6]. Overexpression of HSP70 can help the tumor cells to survive by inhibiting both intrinsic and extrinsic apoptotic pathways [7–9]. Bax, a member of the intrinsic apoptosis pathway and a pro-apoptotic Bcl-2 family, can directly bind to HSP70, which inhibits the activation and translocation of Bax to the mitochondria [10, 11]. The generation of the ROS molecule cytochrome c (cyt-c) is also inhibited. HSP70 can also interact with the death receptors 4 and 5 of the extrinsic apoptotic pathway, which prevents the production of death signaling complexes [12]. Additionally, HSP70 also interacts with apoptosis-inducing factor (AIF) to inhibit apoptosis by caspase-independent mechanisms [12, 13]. HSP70 as an anti-apoptotic protein boosts cell survival in malignant cells [14]. Therefere, HSP70 is involved in regulating caspase activity by blocking both intrinsic and extrinsic apoptotic pathways. The present study hypothesizes that the stress protein HSP70 is released as a response to chemoradiation in SiHa cells. To prove this, the measured HSP70 protein expressions in cisplatin alone, radiation alone, and chemoradiation treated SiHa cells are compared to untreated SiHa cells HSP70 level.

2 Materials and Methodology

The Ethics Approval for the study is granted by the Ethics Committee of Basavatarakam Indo-American Cancer Hospital & Research Institute, Hyderabad, India. The cervical squamous carcinoma SiHa (Human papillomavirus 16 positive) cell line is obtained from the National Centre for Cell Science (NCCS) in Pune, India. The cell line is 100% matched with the morphological features of the original phenotype of the American Type Culture Collection Cells (ATCC) and no contamination is found. For cell culture, the reagents are warmed in a water bath for about 20–30 minutes before the experiment. The cells are then seeded by adding complete media in a labeled 75 cm^2 culture flask. Complete media is prepared by adding 100ml of DMEM media, 10% of FBS and 1 ml of antibiotic. After a gentle shake, the cells are cultivated at 37 °C in sterile humidified ambient conditions with 5% CO_2. The media is replaced every 24 h of incubation to maintain nutritional levels for the developing cells. Upon 24 h of incubation, with the visible nucleus the SiHa cells are grown as elongated spindle shaped adherent cells as shown in Fig. 3 (a). The similar morphology of SiHa cells is observed by the other research group [15].

After obtaining 80% of confluency, 2^{10} number of cells are seeded in 6 well plates for the experiment as shown in Fig. 1. A 6 well plate with round well shape and the

media level of 1 cm height in each well are suggested by the radiation physicist. Wells of the 6 well plates are grouped into two categories with 3 wells each. Wells 1, 3, and 5 are considered the radiation alone group, and the wells 2, 4, and 6 are labeled as cisplatin with radiation (chemoradiation).

After 24 h, the SiHa cells in 2, 4, and 6 wells are induced with 17 μM of cisplatin drug for chemoradiation experiment. All the experiments are carried out in triplicates to avoid any chance of error. Before radiation, the cells in 6 well plates are observed under a microscope to understand the cell conditions. After the cells are ready, the cells are radiated using Elektra synergy linear accelerator in the radiation department of Basavatarakam cancer hospital. The radiation type used in the study is X-rays with a beam energy of 4MV and the technique is static. The dose rate is 25 MV/min. After the plate has been properly adjusted on the table as illustrated in Fig. 2, the cells are exposed to various dosages of radiation (2, 4, 6, and 8 Gy (Gy)). For 2, 4, 6, and 8 Gy, the total MV supplied to cells is 201 MV, 407 MV, 610 MV, and 813 MV, respectively. The cells are brought to the laboratory after they have been irradiation.

Serum starvation procedure is followed for HSP70 protein extraction. For serum starvation the treated and untreated cells are starved in serum-free condition (i.e.., without serum FBS) for 24 h. The HSP70 protein is measured after starvation using an enzyme-linked immunosorbent assay (ELISA). In the present study, the ELISA kit is procured from Cat#: ELH-HSP70-001, Ray Biotech Inc.

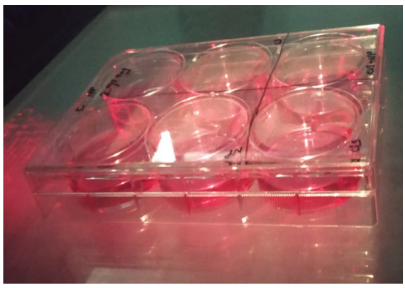

Fig. 1. SiHa cells seeded in 6 well plate

Fig. 2. Adjusting the 6-well plate on the radiation table

The current work also focuses on determining the levels of HSPA1A gene expression in SiHa cells. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) is a housekeeping/reference gene that serves as an endogenous check for continuous expression. The HSPA1A gene expression in SiHa cervical cancer cells treated with different radiation doses is examined using the qRT-PCR technique. The following steps are involved in the investigation of gene expression: firstly, the RNA is isolated from the cells. Secondly, the acquired RNA is then transformed to cDNA (complementary DNA). Finally, transformed cDNA serves as a template strand for qRT-PCR.

3 Results and Discussions

Pre-apoptotic morphological alterations, such as cell swelling and cell disruptions, are observed in treated and untreated SiHa cells. The cells in all the wells are healthy with no visible contamination. Prior to radiation, the cells in wells 1, 3, and 5 appeared to be healthy, active in proliferation, and have ideal morphologic features as shown in Fig. 3 (a). They adhered effectively, mitotic divisions are seen, and the cells had identical spindle and elongated shape. As shown in Fig. 3 (b) The cells in the wells 2, 4, and 6 treated with cisplatin are observed to have cell disruptions.

The polygonal shape is acquired by SiHa cells after being exposed to various radiation doses (2, 6, and 8 Gy) as shown in Fig. 3 c, d, and e. Secondly, the swelling of cells is also noted, which indicates that cellular damage has been initiated in the cells. When cisplatin and radiation (2, 6, or 8 Gy) are administered to treat SiHa cells, there is a greater degree of cell destruction and a greater proportion of round and polygonal-shaped cells. As seen in Fig. 3 f, g, and h certain cells have disturbed cell organelles. In cisplatin combined with radiation treatment, more rounded cells are seen when compared with cisplatin alone Fig. 3 (b). Microscopic images captured pre- and post-treatment are used to monitor the morphological changes of the SiHa cells. An elongated spindle-like structure is observed in the untreated SiHa cells. The SiHa cell morphology is also substantiated by a few other studies [15–17]. When compared to untreated SiHa cells, 2, 6, and 8 Gy radiation-alone and chemoradiation-treated cells showed an irregular polygonal structure. Cell swelling is seen in response to both therapies and is also consistent with other research. Cellular swelling is the initial sign of any cellular damage [17]. Additionally, when compared to cisplatin alone, microscopic images of chemoradiation-treated cells revealed a greater proportion of rounded and disrupted cells. It has been further observed that at 8 Gy radiation combined with cisplatin resulted in more round and disrupted cells. This indicated that cisplatin with 8 Gy radiation is providing effective treatment. Similar results are reported by other research groups [15–22].

HSP70 expression in treated SiHa cells:

In the current work, the expression of HSP70 in SiHa cervical cells treated with radiation alone (2, 4, 6, and 8 Gy) and cisplatin combined with radiation (2, 4, 6, and 8 Gy) is determined and compared with the untreated SiHa cell's HSP70 value. Elisa is used for estimating the HSP70 expression levels in the culture media. Previous studies have shown the release of HSPs into the cultured media by the cells [23, 24].

Using the standard curve the HSP70 value is caluculated from the absorbance obtained from ELISA method. Figure 4 shows the HSP70 in untreated and cisplatin

alone (chemotherapy) treated cells. The Fig. 4 also shows the HSP70 expressed in SiHa cells radiated at different doses (2, 6, and 8 Gy) and cisplatin combined with radiation (2, 6, and 8 Gy). In untreated SiHa cells, HSP70 expression is 17.98 ng/ml. HSP70 levels in 2, 6, and 8 Gy radiation-treated cells are 228, 255, and 308 ng/ml. HSP70 expression in cells treated with cisplatin - alone is 207 ng/ml. HSP70 levels are 276, 320, and 328 ng/ml in cells treated with cisplatin and 2, 6, and 8 Gy radiation, respectively. In the present study, a low expression of HSP70 is expressed by untreated SiHa cells. Low amounts of protein release could be because of the microenvironmental stress experienced by cancer cells, such as hypoxia and starvation. It is referred to as viable HSP70. The viable HSP70 is also recorded in another research study [25].

When compared to untreated SiHa cells, post-chemoradiation HSP70 levels are elevated. Other research teams have validated the findings demonstrating an increase in post-treatment HSP70 expression when compared with untreated cancer cells N1 H3 T3 cells radiated with 3.81 Gy [26], c3H 10T ½ cells [27], prostate cancer cell lines, and also in xenografts [28]. Recent investigations have revealed that HSP70 expression is

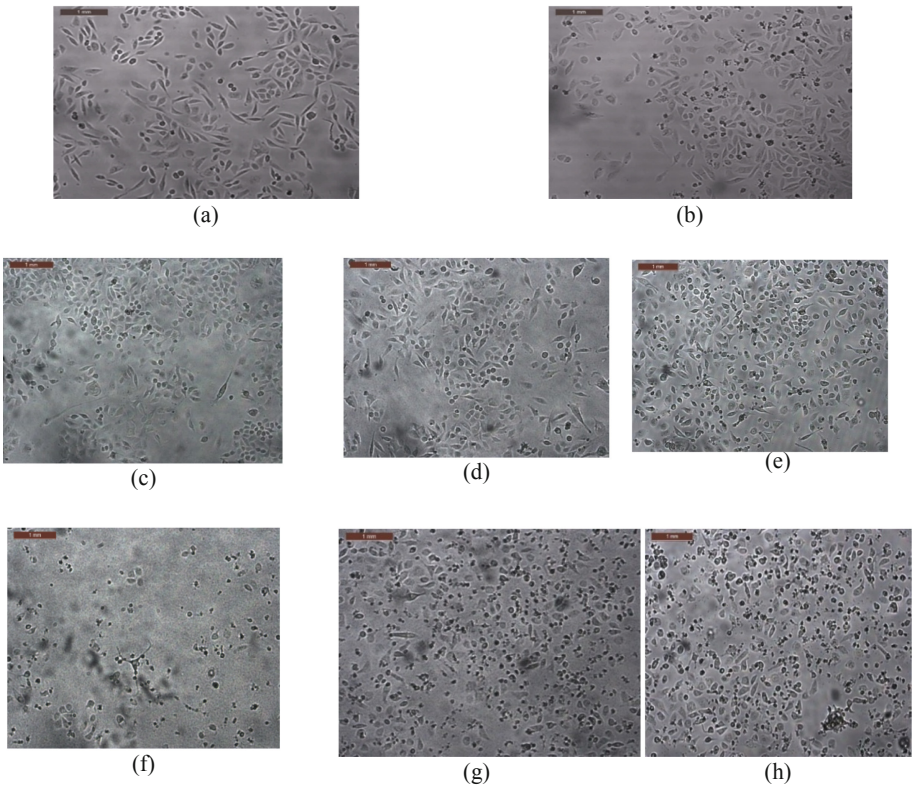

Fig. 3. Morphological characteristics of SiHa cells (a) untreated SiHa cells, (b) SiHa cells treated with cisplatin, (c, d, e) SiHa cells post-radiation at 2, 6, and 8 Gy, (f, g, h) SiHa cells post-chemoradiation at 2, 6, and 8 Gy

extremely low in normal cells but quite high in a variety of cancers [29, 30]. According to a study, radiation-induced cellular damage results in the induction of the HSP70 protein [31]. If HSP70 expression in the untreated SiHa cells is assumed to be a unit (17.98 ng/ml), radiation alone-treated cells indicated a rise in the protein expression approximaly 12, 14, and 17-fold at 2, 6, and 8 Gy, respectively.

In the present investigation, chemoradiated cells showed elevated levels of HSP70 expression than radiation-alone treated cells. Studies revealed that other cancer cell lines treated with different chemotherapeutic drugs also exhibited a rise in the HSP70, doxycycline-treated MCF-7 [32], HT-29 treated with 5-FU [33]. Cervical cancer treatment combined with radiation and chemotherapy showed to improve the treatment outcome [34]. Chemotherapy affects the cell cycle stages and promotes cell death by increasing the cellular damage induced by radiation [35]. The cervical cancer patients receiving chemotherapy and radiation therapy showed a reduction in the mortality rate by 30–50%. Similarly, considering the HSP70 expression of untreated SiHa cells as a unit upregulation of HSP70 is observed in chemoradiation treated cells approximately 15, 17, and 18-fold at 2, 6, and 8 Gy, respectively. Compared to the radiation-alone or cisplatin-alone cells, the HSP70 levels post-chemoradiation are upregulated. The microscopic visualizations supported the ELISA results. The HSP70 protein released during chemoradiation is thereby found to be linearly proportional to the dosage radiations (2, 6, and 8 Gy), and the observations from the images also supported the data.

The increase in anti-apoptotic HSP70 protein expression can be closely correlated with the triggering of the apoptotic pathway and the survival of malignant cells [36–38]. Further studies showed that SiHa cell survival is radiation dose dependent. SiHa cell viability is significantly decreased as radiation doses from 2 to 8 Gy are increased [39]. Other cervical cancer cell lines HeLa, C33A, and CaSki also confirmed lower survival at 2, 4, 6, and 8 Gy dose-dependent radiations [40, 41].

The reason for the upregulation of post-treatment HSP70 levels is explained in the following discussion. Radiation initiates apoptosis by inducing damage to the vital cellular

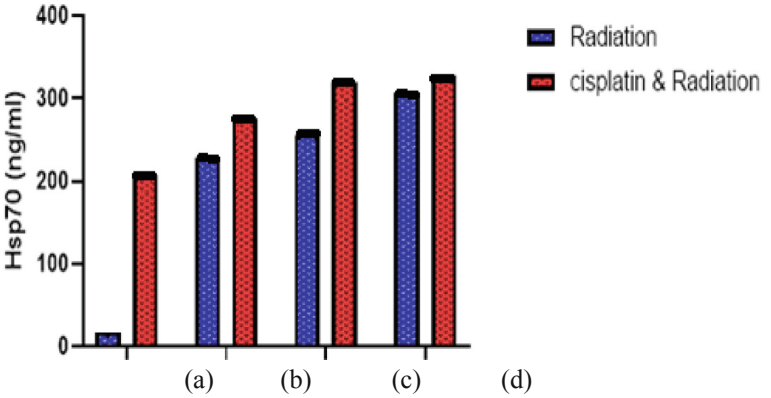

Fig. 4. HSP70 levels of a) untreated SiHa cells and cisplatin alone treated SiHa cells (b) SiHa treated at 2 Gy (c) SiHa cells treated at 6 Gy (d) SiHa cells treated at 8 Gy

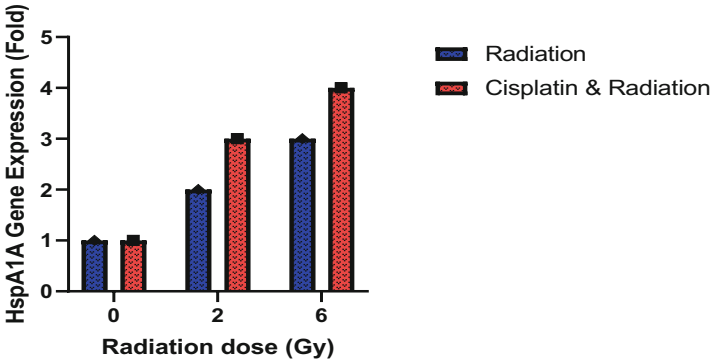

Fig. 5. HSPA1A gene Fold change in chemoradiated SiHa cells

organelles like DNA (i.e., single, and double-strand breaks) [42], endoplasmic reticulum [43], mitochondria, ribosomes [28], lysosomes, plasma membrane. When radiation causes cellular damage, reactive oxygen species are increased. Both in vitro and in vivo, the generation of the ROS molecule cytochrome c (cyt-c) and death receptors alter the permeability of the mitochondrial membrane to trigger apoptosis [44]. HSP70 as an anti-apoptotic protein inhibits the apoptotic pathway [45]. HSP70 inhibits cell death at different points namely cytochrome c and death receptors [46], pro-apoptotic Bcl-2 family [47], Bax [47], and caspases [48].

HSPA1A gene expression in treated SiHa cells:

For HSPA1A gene expression studies the ratio of absorbance at 260nm and 280nm is between 2.0 and 2.2 for all the samples which indicates that the RNA extracted is pure. The HSPA1A gene is perfectly amplified at 60 ℃. The gene expression results are studied as fold change. Initially, the fold value of untreated SiHa cells is standardized as 1. The fold changes obtained at 2 Gy and 6 Gy radiation and chemoradiation are shown in Fig. 5. In 2 Gy radiated cells HSPA1A gene is upregulated by 1-fold and in chemoradiated cells, the fold change is 2. In 6 Gy radiated cells HSPA1A gene is upregulated by 2-fold and in chemoradiated cells, the HSPA1A gene is upregulated by 3-fold.

In the current work, in addition to the increase in HSP70 protein expression, a progressive rise in HSPA1A gene expression level with an increase in radiation dosage is detected. In SiHa cells the HSP70 gene expression is upregulated by 3-fold in chemoradiated cells when compared with the untreated SiHa cells. The other studies also showed the upregulation of HSP70 gene expression post-radiation [4, 49, 50] which establishes the present study results.

4 Conclusion

In this chapter, the expression of HSP70 is estimated in radiation alone, cisplatin alone, and chemoradiation treated SiHa cells. According to the results, chemoradiation induces cellular damage and activates the cytoprotective mechanism in cells as a response to the

treatment. SiHa cervical cancer cells treated with chemoradiation showed overexpression of HSP70 compared to radiation alone and cisplatin alone. A dose dependent (ie. 2, 6, 8 Gy) increase of HSP70 is observed. The HSP70 protein levels and image observations supported the following hypothesis that chemoradiation triggers the release of HSP70. The impact of radiation on the cellular damage is radiation dose-dependent, and when compared to radiation alone, chemoradiation has a greater impact on the cell. The overexpression of HSP70 might directly affect the clinical outcome of the patient. The study findings might have significant future clinical relevance, in monitoring the clinical outcome of chemoradiation or cancer prognosis in cervical cancer patients.

References

1. Sung H, Ferlay J, Seigel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin.;71:209–49, (2021).
2. Bhatla, N., Meena, J., Kumari, S. *et al.* Cervical Cancer Prevention Efforts in India. *Indian J Gynecol Oncolog* **19**, 41, https://doi.org/10.1007/s40944-021-00526-8, (2021).
3. Segeritz, Charis-P., and Ludovic Vallier. "Cell Culture: Growing Cells as Model Systems In Vitro." Basic Science Methods for Clinical Researchers: 151– 172. doi:https://doi.org/10.1016/B978-0-12-803077-6.00009-6, (2017).
4. Turrini .E, E. Catanzaro, M. G. Muraro et al., "Hemidesmus indicus induces immunogenic death in human colorectal cancer cells," Oncotarget, vol. 9, no. 36, pp. 24443–24456, (2018).
5. Schmitt E, Gehrmann M, Brunet M, Multhoff G, Garrido C. Intracellular and extracellular functions of heat shock proteins: Repercussions in cancer therapy. J Leukoc Biol; 81: 15–27 (2007).
6. Moradi-Marjaneh, .R, M. Paseban, and M. Moradi Marjaneh, "Hsp70 inhibitors: implications for the treatment of colorectal cancer," IUBMB Life, vol. 71, no. 12, pp. 1834–1845, (2019).
7. Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M. "Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases", EMBO J. 1998;17(21):6124–34. doi:https://doi.org/10.1093/emboj/17.21.6124, (1998)
8. Jaattela M. "Escaping cell death: survival proteins in cancer", Exp Cell Res. 1999;248(1):30–43, (1999).
9. Nylandsted J, Gyrd-Hansen M, Danielewicz A, Fehrenbacher N, Lademann U, Hoyer-Hansen M, et al. "Heat shock protein 70 promotes cell survival by inhibiting lysosomal membrane permeabilization", J Exp Med. 2004;200(4):425–35, (2004).
10. Stankiewicz AR, Lachapelle G, Foo CP, Radicioni SM, Mosser DD. "HSP70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation", J Biol Chem. 2005;280(46):38729–39, (2005).
11. Gotoh T, Terada K, Oyadomari S, Mori M. "hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria", Cell Death Differ. 2004;11(4):390–402. doi:https://doi.org/10.1038/sj.cdd.4401369, (2004)
12. Guo F, Sigua C, Bali P, George P, Fiskus W, Scuto A, et al. "Mechanistic role of heat shock protein 70 in Bcr-Abl-mediated resistance to apoptosis in human acute leukemia cells", Blood. 2005;105(3):1246–55. doi:https://doi.org/10.1182/blood-2004-05-2041, (2005).
13. Demidenko, Z.N., Vivo, C., Halicka, H.D., Li, C.J., Bhalla, K., Broude, E.V. & Blagosklonny, M.V. "Pharmacological induction of Hsp70 protects apoptosis-prone cells from doxorubicin: comparison with caspase-inhibitor- and cycle-arrest-mediated cytoprotection", *Cell Death and Differentiation,* **13**(9), 1434-1441, (2006).

14. Kumar S, Stokes J, III, Singh UP, Scissum Gunn K, Acharya A, Manne U, Mishra M. "Targeting Hsp70: A possible therapy for cancer", Cancer Lett. 2016;374:156–166. doi: https://doi.org/10.1016/j.canlet.2016.01.056, (2016).
15. Sujata & Sharma, Himani & Sharma, Chandresh & Jayaram, Hiremagalur & Singh, Neeta. "Apoptotic signaling induced by Tiazofurin-an in vitro study", Gene therapy & molecular biology. 10. 199–206, (2006).
16. Yan L, Liu X, Yin A, Wei Y, Yang Q and Kong B: Huaier "aqueous extract inhibits cervical cancer cell proliferation via JNK/p38 pathway", Int J Oncol 47: 1054-1060, (2015)
17. L,v,H., Jin, S., Zou, B. *et al.* "Analyzing the whole-transcriptome profiles of ncRNAs and predicting the competing endogenous RNA networks in cervical cancer cell lines with cisplatin resistance", *Cancer Cell Int* **21**, 532 https://doi.org/10.1186/s12935-021-02239-6, (2021).
18. Matthew A. Wallig, Evan B. Janovitz, "Morphologic Manifestations of Toxic Cell Injury", Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition)
19. Ren B, Luo S, Xu F, Zou G, Xu G, He J, Huang Y, Zhu H, Li Y. "The expression of DAMP proteins HSP70 and cancer-testis antigen SPAG9 in peripheral blood of patients with HCC and lung cancer", Cell Stress Chaperones. 2017 Mar;22(2):237–244. doi: https://doi.org/10.1007/s12192-016-0758-5. Epub 2016 Dec 27. PMID: 28028759; PMCID: PMC5352597, (2016)
20. Xu Y, Wang C, Li Z. "A new strategy of promoting cisplatin chemotherapeutic efficiency by targeting endoplasmic reticulum stress", Mol Clin Oncol. 2014 Jan;2(1):3–7. doi: https://doi.org/10.3892/mco.2013.202. Epub Oct 18. PMID: 24649299; PMCID: PMC3916193, (2013).
21. Yonika Arum Larasati, Dyaningtyas Dewi Pamungkas Putri, Rohmad Yudi Utomo, Adam Hermawan, Edy Meiyanto. "Combination of Cisplatin and Cinnamon Essential Oil Inhibits HeLa Cells Proliferation through Cell Cycle Arrest", J App Pharm Sci, 2014; 4 (12): 014–019, (2014).
22. Jin, B., Kong, W., Zhao, X., Chen, S., Sun, Q., Feng, J., Song, D., & Han, D "Substrate stiffness affects the morphology, proliferation, and radiosensitivity of cervical squamous carcinoma cells", *Tissue and Cell*, *74*, 101681. https://doi.org/10.1016/j.tice.2021.101681, (2022).
23. Kuncic Z, Lacombe S. "Nana2oparticle radioenhancement: principles, progress and application to cancer treatment", Phys Med Biol. 2018 Jan 9;63(2):02TR01. doi: https://doi.org/10.1088/1361-6560/aa99ce. PMID: 29125831, (2018)
24. Broquet .A.H, G. Thomas, J. Masliah, G. Trugnan, M. Bachelet, "Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release", J. Biol. Chem, 278 (2003), pp. 21601–21606, (2003)
25. Lancaster GI, Febbraio MA: "Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins", *J Biol Chem* 2005, 280: 23349-23355. https://doi.org/10.1074/jbc.M502017200, (2005)
26. Lee SJ, Choi SA, Lee KH, Chung HY, Kim TH, Cho CK, Lee YS. "Role of inducible heat shock protein 70 in radiation-induced cell death. Cell Stress Chaperones", Jul;6(3):273–81. doi: https://doi.org/10.1379/1466-1268(2001)006<0273:roihsp>2.0.co;2. PMID: 11599569; PMCID: PMC434409 , (2001)
27. Calini V, Urani C, Camatini M. "Overexpression of HSP70 is induced by ionizing radiation in C3H 10T1/2 cells and protects from DNA damage", Toxicol In Vitro. 2003 Oct-Dec;17(5–6):561–6. doi: https://doi.org/10.1016/s0887-2333(03)00116-4. PMID: 14599445, (2003)
28. Tang D, Khaleque MA, Jones EL, Theriault JR, Li C, Wong WH, Stevenson MA, Calderwood SK. "Expression of heat shock proteins and heat shock protein messenger ribonucleic acid in human prostate carcinoma in vitro and in tumors in vivo", Cell Stress Chaperones. 2005 Spring;10(1):46–58. doi: https://doi.org/10.1379/csc-44r.1. PMID: 15832947; PMCID: PMC1074571, (2005)

29. Yoshidomi K, Murakami A, Yakabe K, Sueoka K, Nawata S, Sugino N. "Heat shock protein 70 is involved in malignant behaviors and chemosensitivities to cisplatin in cervical squamous cell carcinoma cells", *J Obstet Gynaecol Res.* 2014;40:1188–1196. doi: https://doi.org/10.1111/jog.12325, (2014)
30. Daugaard M, Rohde M, Jäättelä M. "The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions", *FEBS Lett.* 2007;581:3702–3710. doi: https://doi.org/10.1016/j.febslet.2007.05.039, (2007)
31. Murakami, N., Kühnel, A., Schmid, T.E. *et al.* "Role of membrane Hsp70 in radiation sensitivity of tumor cells", *Radiat Oncol* **10,** 149 (2015). https://doi.org/10.1186/s13014-015-0461-1, (2015)
32. Barnes, J. A., Dix, D. J., Collins, B. W., Luft, C., & Allen, J. W. "Expression of inducible Hsp70 enhances the proliferation of MCF-7 breast cancer cells and protects against the cytotoxic effects of hyperthermia", Cell stress & chaperones, 6(4), 316–325. https://doi.org/10.1379/1466-1268(2001)006<0316:eoihet>2.0.co;2, (2001).
33. Grivicich I, Regner A, Zanoni C, Correa LP, Jotz GP, Henriques JA, Schwartsmann G, da Rocha AB. "Hsp70 response to 5-fluorouracil treatment in human colon cancer cell lines", Int J Colorectal Dis. 2007 Oct;22(10):1201–8. doi: https://doi.org/10.1007/s00384-007-0307-x. Epub 2007 Mar 28. PMID: 17390142, (2007)
34. Trimble E, Gius D, Harlan LC: "Impact of NCI announcement upon use of chemoradiation for women with cervical cancer", J Clin Oncol 25:283s, 2007 (suppl; abstr 5537) , (2007)
35. Dasari, S.; Tchounwou, P. B. "Cisplatin in cancer therapy: molecular mechanisms of action", Eur J Pharmacol, 740: 364–78; ISSN 0014- 2999, (2014).
36. Zhang Z, Shao Z, Xiong L, Yang S. "Inhibition of autophagy enhances cisplatin-induced apoptosis in the MG63 human osteosarcoma cell line", *Oncol Lett.* 2015;10:2941–2946. doi: https://doi.org/10.3892/ol.2015.3692, (2015)
37. Seki K, Yoshikawa H, Shiiki K, Hamada Y, Akamatsu N, Tasaka K. "Cisplatin (CDDP) specifically induces apoptosis via sequential activation of caspase-8, -3 and -6 in osteosarcoma", *Cancer Chemotherphy Pharmacol.* 2000;45:199–206. doi: https://doi.org/10.1007/s002800050030, (2000)
38. Goloudina AR, Demidov ON, Garrido C. "Inhibition of HSP70: A challenging anti-cancer strategy", *Cancer Lett.* 2012;325:117–124. doi: https://doi.org/10.1016/j.canlet.2012.06.003, (2012)
39. Liu, Gui-Feng & Zhang, Shu-Hua & Li, Xue-Feng & Cao, Li-Yan & Fu, Zhan- Zhao & Yu, Shao-Nan. "Overexpression of microRNA-132 enhances the radiosensitivity of cervical cancer cells by down-regulating Bmi-1", Oncotarget. 8. https://doi.org/10.18632/oncotarget.20358, (2017)
40. Fellinger H, Stangl S, Hernandez Schnelzer A, Schwab M, Di Genio T, Pieper M, Werner C, Shevtsov M, Haller B, Multhoff G. "Time- and Dose-Dependent Effects of Ionizing Irradiation on the Membrane Expression of Hsp70 on Glioma Cells. Cells", Apr 8;9(4):912. doi: https://doi.org/10.3390/cells9040912. PMID: 32276468; PMCID: PMC7226755, (2020)
41. Su, Wen-Hong & Chuang, Pei-Chin & Huang, Eng-Yen & Yang, Kuender. "Radiation-Induced Increase in Cell Migration and Metastatic Potential of Cervical Cancer Cells Operates Via the K-Ras Pathway. The American journal of pathology". 180. 862–71. https://doi.org/10.1016/j.ajpath.2011.10.018, (2011).
42. van Meir H, Nout RA, Welters MJ, et al. "Impact of (chemo)radiotherapy on immune cell composition and function in cervical cancer patients", Oncoimmunology;6(2):e1267095. Published 2016 Dec 23. doi:https://doi.org/10.1080/2162402X.2016.1267095, (2016).
43. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C, "Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways" Biochem Biophys Res Commun 304:505–512, (2003).

44. Kamer, S., Ren, Q. & Dicker, A.P. "Differential radiation sensitization of human cervical cancer cell lines by the proteasome inhibitor velcade (bortezomib, PS-341)", *Arch Gynecol Obstet* **279**, 41–46, https://doi.org/10.1007/s00404-008-0667-7, (2009).
45. Jagadish, N., Parashar, D., Gupta, N. *et al.* "Heat shock protein 70–2 (HSP70-2) is a novel therapeutic target for colorectal cancer and is associated with tumor growth", *BMC Cancer* **16**, 561 (2016). https://doi.org/10.1186/s12885-016-2592-7, (2016)
46. Gupta N, Jagadish N, Surolia A, Suri A. Erratum: "Heat shock protein 70–2 (HSP70–2) a novel cancer testis antigen that promotes growth of ovarian cancer", Am J Cancer Res. 2020 Jun 1;10(6):1919–1920. Erratum for: Am J Cancer Res. 2017 Jun 01;7(6):1252–1269. PMID: 32642300; PMCID: PMC7339280, (2017)
47. Stark, Holger & Zivkovic, Aleksandra, "HPV Vaccination: Prevention of Cervical Cancer in Serbia and in Europe", Acta Facultatis Medicae Naissensis. 35. 5–16. https://doi.org/10.2478/afmnai-2018-0001, (2018).
48. Aghdassi A, Phillips P, Dudeja V, Dhaulakhandi D, Sharif R, Dawra R, Lerch MM, Saluja A. "Heat shock protein 70 increases tumorigenicity and inhibits apoptosis in pancreatic adenocarcinoma", Cancer Res, Jan 15;67(2):616–25. doi: https://doi.org/10.1158/0008-5472.CAN-06-1567. PMID: 17234771, (2007).
49. Rosa, S., Connolly, C., Schettino, G. et al. "Biological mechanisms of gold nanoparticle radiosensitization", Cancer Nano **8,** 2 (2017). https://doi.org/10.1186/s12645-017-0026-0, (2017)
50. De Andrade WP, Da Conceição Braga L, Gonçales NG, Silva LM, Da Silva Filho AL. "HSPA1A, HSPA1L and TRAP1 heat shock genes may be associated with prognosis in ovarian epithelial cancer", Oncol Lett. 2020 Jan;19(1):359–367. doi: https://doi.org/10.3892/ol.2019.11095. Epub 2019 Nov 14. PMID: 31897148; PMCID: PMC6923843, (2019)