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DHRS2 is a potential marker of breast cancer metastasis☆

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ABSTRACT

Background: Although breast cancer treatment success has increased, biomarkers are still important. The dehydrogenase/reductase DHRS2 has a role in the metabolic and survival activities of cells. DHRS2 directly binds MDM2 and inhibits the cellular processes of p53. Our study aimed to examine cellular response to inhibit DHRS2 gene expression in the breast cancer cell line MCF7.

Methods and results: The RNAi suppressed DHRS2 expression has been investigated in the breast carcinoma cell line MCF7 and, different study groups were formed to observe the impact. Afterward, an apoptotic marker was applied to the groups for measuring the amount of apoptotic and necrotic cells by flow cytometer. Cells were stained with trypan blue for cell viability, and we used an MTT assay for cellular proliferation measurement. Finally, migration experiments were performed using the wound healing model, and their gap width was calculated with the ImageJ program. Comparing to the non-transfected cells (NTC), the apoptotic cell number of non-targeting (NT) and DHRS2 siRNA (si-DHRS2) transfected groups had less quantity. While there was no critical difference in proliferation and viability, DHRS2 deficient cells gave the lowest closure rate than control groups in migration tests. The gene silencing rates of all groups were controlled by quantitative PCR.

Conclusions: The migration assay results of DHRS2 inhibition, the gene popularity is increasing day by day in the literature, will be pioneers for metastasis studies. It is also usual that cell apoptosis and proliferation, which have many regulatory mechanisms, are not critically affected by the absence of DHRS2.

1. Introduction

Every year hundreds of thousands of women are diagnosed as breast cancer patients, and many women die because of the disease. Molecular genetics of this heritability cancer is known with the key lines. Thus, more detailed studies are needed to understand the cellular mechanism of breast cancer.

SDR family proteins have many isoforms in different tissues and take other cellular processes as a catalyzer. Their substrates are variable, and they convert steroid hormones, retinoids, and prostaglandins from an active form to an inactive form. SDR enzymes are determinative on critical functions like cell cycle control because of their effects on regulatory molecules (Oppermann et al., 2001; Bray et al., 2009; Pellegrini et al., 2002).

SDR family member DHRS2 (Hep27) is located long arm of chromosome 14, and its transcript has nine exons. The human DHRS2 gene is expressed in the liver where it identified, parotid gland, placenta, ovary, breast, and testis tissue (Pellegrini et al., 2002; Shafqat et al., 2006). Still, except for dendritic cells, there is no expression of DHRS2 in monocytes and monocyte-derived macrophages, according to

Abbreviations: DHRS2, dehydrogenase/reductase SDR family member 2; RNAi, RNA interference; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; siRNA, small interfering RNA; NTC, non-transfected cells; NT, non-targeting siRNA applied cells; Si-DHRS2, DHRS2 siRNA applied cells; PCR, Polymerase Chain Reaction; SDR, short-chain dehydrogenases/reductases family; qRT-PCR, quantitative real time PCR; 7AAD, 7-aminoactinomycin D; RNA, ribonucleic acid; cDNA, complementary DNA; ER, estrogen receptor.

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publications (Heinz et al., 2002). DHRS2 protein is found in the mitochondrial matrix, nucleus, and cytoplasm. Like other SDR enzymes, it blocked signal molecules before they reached their nuclear receptors (Monge et al., 2009; Gabrielli et al., 1995).

Besides roles in metabolic processes, DHRS2 has a tumor suppressor feature under inhibition of MDM2 by binding the central domain of the protein. Also, DHRS2 contributes to regulating p53 by blocking the E3 ubiquitinase activity of MDM2. Besides the tumor suppressor activity, DHRS2 has a vital role in lipid metabolism and reduces a-dicarbonyl compounds for alleviating cellular oxidative stress damage. DHRS2 takes part in peroxisomal transport (Honda et al., 1997; Geng et al., 2013; Pei et al., 2012; Chao, 2015).

Our study shows the DHRS2 effects on ER+ breast cancer cell line MCF7 in different methods.

2. Material and methods

The effects of DHRS2 were investigated in the MCF7 (ATCC® HTB-22™) cell line using siRNA-mediated gene expression silencing, which is one of the widespread RNA interference (RNAi) applications. In the beginning, we optimized the optimal dose and time interval for the cells. According to the quantification results of qRT-PCR, 25 nM siRNA (Dharmacon™ SMARTPool™) and 24-hour intervals provided the highest efficiency.

All cells were plated in the culture dishes simultaneously and exposed to the same agents to elution the batch effect. We formed three groups as DHRS2 siRNA (si-DHRS2), non-targeting siRNA (NT), and non-transfected cells (NTC), and these groups were studied in duplicates.

After the incubation times, RNA isolations of experimental groups were performed using PureLink® RNA Mini Kit following the manufacturer's instructions. Total RNA was converted into cDNA with a reverse transcription method using the High Fidelity Kit® cDNA Synthesis Kit, which was used to determine the expression ratio of the target gene via qRT-PCR. The expression level of the DHRS2 gene was determined by comparing it with the housekeeping gene TBP.

At the end of the 24- and 48-hour incubation periods of the cells, they were removed from the surface of 24-well culture dishes by trypsin-EDTA, and then the cells were washed with PBS after harvesting transferred to flow tubes. A small part of the harvested cells was stained with trypan blue and counted in the Vi-CELLXR device. The other cells were divided into two groups, stained with PE Annexin V/7AAD (BD Pharmingen™) following the manufacturer's protocol and the unstained groups. One hundred thousand cells were counted with BD FACSCalibur™ flow cytometer. Cells were seeded in 96-well plates for cell proliferation assay. At the end of the 24, 48, and 72-hour incubation times, a metabolic indicator tetrazolium dye MTT was applied to cells, and assay protocol was carried out. Plates were read at 540 nm in a Multiskan Spectrophotometer.

When the cells were seeded into 6-well plates with 90–100% confluent, we drew a straight line with the 200 μl pipette tip for the wound-healing experiment. RNAi application was performed, and we photographed gap closure intervals at certain times. A 4× objective lens was used in the light microscope to take photographs. Gap width measured using the ImageJ program.

The complete statistical analysis and graph drawings are done with GraphPad Prism version 8.0.2.

3. Results

Three different doses of siRNA were tried on the MCF7 cell line. 25 nM, 50 nM, and 100 nM were tested for efficiency, and we continued experiments with 25 nM.

24 and 48-hour experimental groups were formed for ideal siRNA activity on MCF7 cells, which has a doubling time of 29 h, and 95% suppressed DHRS2 expression at the end of 24 h. Expression data were given in Table 1.

Table 1
qRT-PCR expression results of study groups. si-DHRS2 MCF7 cells had the lowest expression of DHRS2 at the end of the 24 h.

	si-DHR6269S2	NT	NTC
24 h	6,22E−06	7,02E−04	4,29E−04
48 h	7,86E−02	1,46E−01	4,12E−01

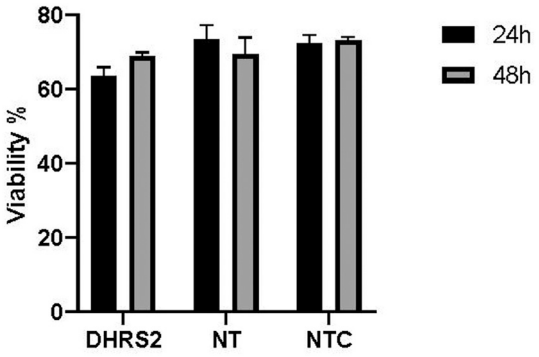


Fig. 1. Viable cell counts presented with columns according to time intervals.

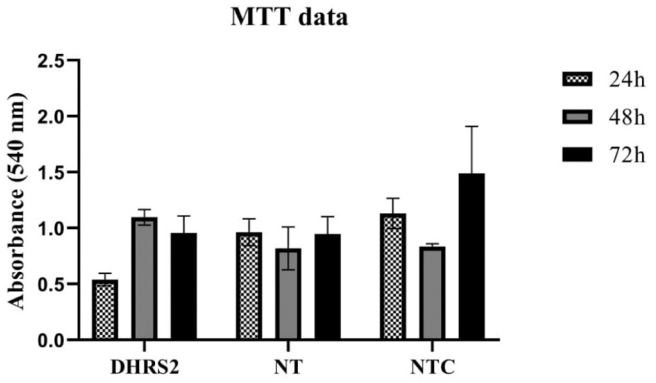


Fig. 2. Study groups were measured at three different time intervals for proliferation determination.

3.1. Cell viability

The viability of si-DHRS2 cells was found to be lower in the 24-hour group compared to the 48-hour. The viable cell counts of transfected groups were less than NTC at the end of the 48th hour (Fig. 1). Based on NTC, we can say that this situation resulted from the decrement of the transfection agent and siRNA activity.

3.2. Cell proliferation

We applied MTT assay to cells in three-time intervals. The most remarkable difference between the control groups and the si-DHRS2 group was observed 24 h (reduction was 50%, shown in Fig. 2). However, with the decreased siRNA activity, the si-DHRS2 group control cells began behaving like control groups shown in Fig. 3 in 48 and 72 h.

3.3. Cell apoptosis

The PE Annexin V staining is a marker for early apoptotic cells. Therefore, since the early apoptotic and late apoptotic cannot be distinguished, we compared the cells as early apoptotic and late

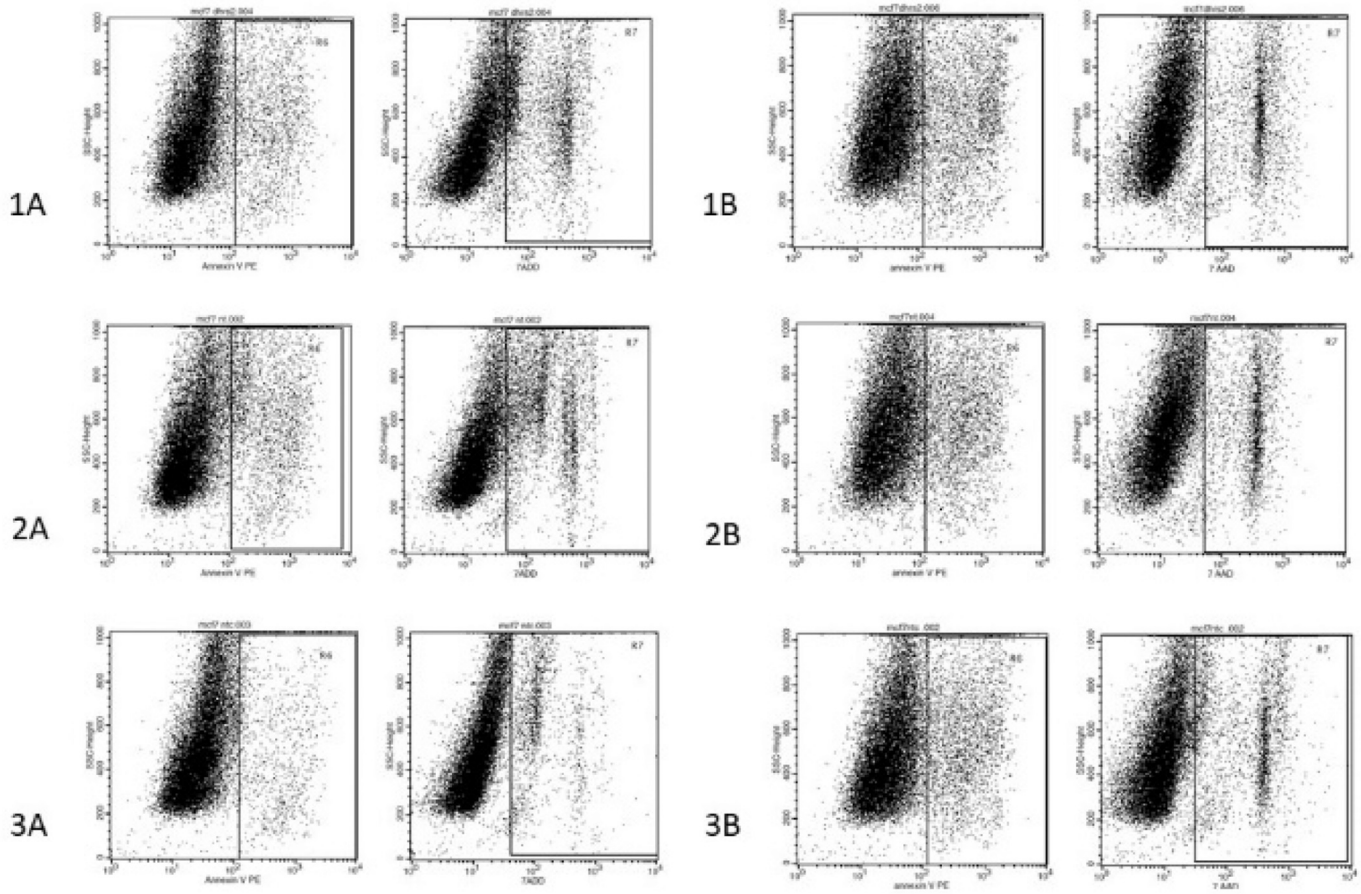


Fig. 3. Flow cytometer results of study groups. Annexin V and 7AAD applications were compared at 24 h and 48 h. (1-si-DHRS2, 2-NT, 3-NTC, A-24hr, B-48hr.)

Table 2
Flow cytometer results are given at the percentage of the counted cells after gated. Two groups have compared each other at 24 and 48-h.

		si-DHRS2	NT	NTC
Early apoptosis	24 h	9,04	13,18	5,39
	48 h	15,91	15,97	14,33
Late apoptosis & necrosis	24 h	28,10	21,25	13,82
	48 h	12,87	13,06	19,75

apoptotic/necrotic cells. The si-DHRS2 cells and control group data are given in Table 2 and Fig. 3. We observed no difference between the si-DHRS2 cells with the control groups between the NT siRNA-treated cells. Compared to NTC, the apoptosis rates of transfected cells were found to be higher. The highest percentage of late apoptotic/necrotic cells was measured in si-DHRS2 cells at the 24th hour.

3.4. Cell migration

Transfected cells were photographed at 0, 15, 24, 36, and 48 h before the wound, using the ProgRes CapturePro v2.8.8 program. The wound closure rate was found to be lower in si-DHRS2 cells compared to control groups. According to the measurements obtained from the Image J program, 51.2% closure was calculated in the si-DHRS2 experimental group. In cells treated with NT siRNA, we detected an acceleration in the closure after 24 h. The NT group, which we think that the effect of the transfection agent is reduced, and the cells are free from gene suppression, showed similarities with the NTC. According to the calculations, 76.1% of wound closure was observed in NT cells. Unsurprisingly, NTC had the highest closure rate (88%) that were not exposed to any agent or chemical proliferated in harmony with the 29-hour division time. Images were presented in Fig. 4.

4. Discussion

Besides having many subtypes, breast cancer differs in its molecular mechanisms. For this reason, studies are aimed at finding possible mechanisms of carcinogenesis and new molecular drug markers.

In this study, cellular changes in the MCF7 cell line with wild-type p53 expression and ER+ in the absence of the DHRS2 gene product referred to as tumor suppressor was investigated. DHRS2 is an NADPH-dependent dehydrogenase/reductase enzyme. Substrates for this enzyme include steroid hormones and cytotoxic dicarbonyl intermediates (Pellegrini et al., 2002; Monge et al., 2009). DHRS2 preprotein, a mitochondrial transport signal, becomes mature after being cut in the mitochondria and returns to the nucleus (Gabrielli and Tofanelli, 2012). DHRS2 binds to the central domain of MDM2 in the nucleus and inhibits the E3 ubiquitinase activity of MDM2, thus providing p53 stabilization in the cell (Deisenroth et al., 2010). DHRS2 has been reported to play a role in p53 regulation, cell cycle, apoptosis, and drug resistance in carcinoma cells. It was observed that DHRS2 gene expression level increased in cells treated with histone deacetylase inhibitors, and apoptosis rates are increased in these cells (Han et al., 2020). However, many studies have emphasized that DHRS2 contributes to cell apoptosis by increasing p53 and cleaved-PARP in the cell by inhibiting MDM2 (Fang et al., 2016).

DHRS2 transcription is regulated by oncogenic proteins such as HOXA13, c-MYB, a-MYB, and ERM/ETV5 (Monge et al., 2009; Deisenroth et al., 2010; Han et al., 2018; Lei et al., 2004). Therefore, it would be beneficial to think that DHRS2 might be involved more than a single

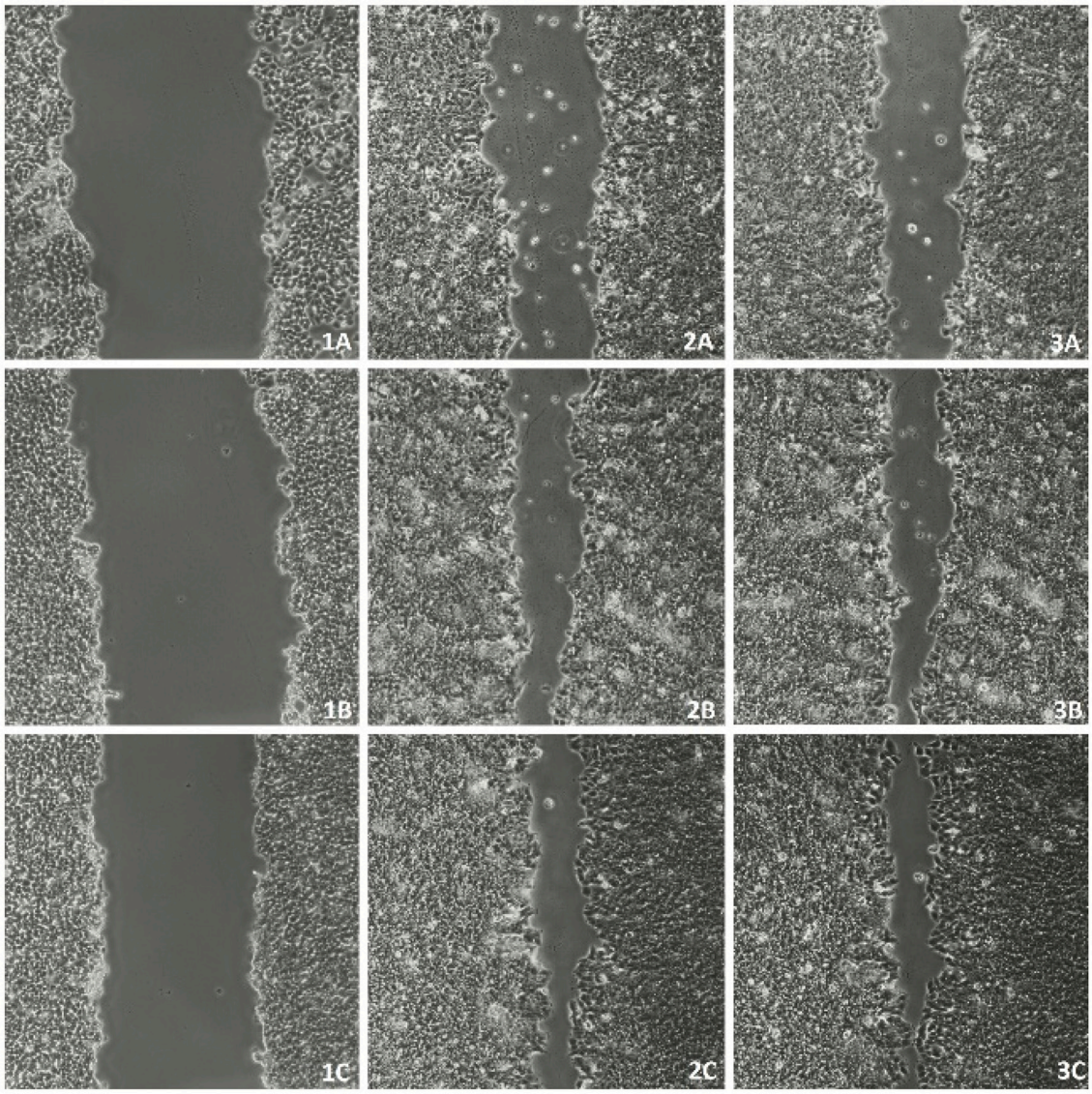


Fig. 4. Light microscopy images present gap closure at different time intervals. (Numbers represent time, letters for study groups. 1-0 h, 2-24 h, 3-48 h; A-si-DHRS2, B-NT, C-NTC.)

cellular pathway.

According to our results, DHRS2 did not significantly impact cell viability, proliferation, or apoptosis in knocked-down cells than control groups but had an opposite effect on cell migration, unlike reported (Zhou et al., 2018). DHRS2 can explain the decrease in cell proliferation, and viability is higher in carcinoma cells than normal cells (Pellegrini et al., 2002; Li et al., 2019; Yuan et al., 2020). In other words, DHRS2 has an inducing effect on the proliferation of cancer cells. Another study reported that DHRS2 and c-MYB were high in the ER+ luminal A breast cancer subtype (Deisenroth et al., 2010). DHRS2 is a dehydrogenase/reductase enzyme that also functions in lipid metabolism (Wang et al., 2013; Luo et al., 2019). Lipid metabolism is one of the energy metabolisms used by cancer cells to escape glycosylation. Since the lack of DHRS2 will also decrease metabolic activities, it is an expected result that cellular proliferation will reduce, and our MTT experiment results were accordant with this expectation.

In apoptosis assays, the apoptosis rates of si-DHRS2 cells were found similar to control cells. However, the highest rate in late apoptotic/necrotic cells was observed in the si-DHRS2 group. These apoptotic cell percentages can be explained in two ways. One option, cells escaped from apoptosis by suppressing p53 with increased MDM2 activity in the absence of DHRS2, and as another option, necrosis occurred due to increased stress after siRNA application. It is known that DHRS2 is protective against oxidative stress and protects the cells from damage by eliminating the reactive products formed after glucose reperfusion by the increase of its expression in cells (Shafqat et al., 2006; Crean et al., 2012).

A significant cell migration reduction appeared in DHRS2 deficient MCF7 cells. It was reported that DHRS2 increased the invasion and migration of cancer cells, and metastasis slowed down in the DHRS2 knocked down HCT116 cell line treated with Oxaliplatin (Li et al., 2019; Zhou et al., 2020).

As a limitation of the study, the gene DHRS2 we examined in the MCF7 cell line is not functionally studied in breast tissue. Transcript variants were found in the qRT-PCR analysis, and commercially gotten siRNA may not be efficient in silencing after 24 h. Another limitation is that discussing the gene function was done with the other cell types because there is little information about gene function in the literature.

5. Conclusion

The DHRS2 gene shows functions as a reducer of cell migration in the

MCF7 cell line. Further studies are needed to reveal the role of DHRS2 in breast cancer to enlarge the information about this gene.

CRediT authorship contribution statement

Burcu Salman Yaylaz: Conceptualization, Validation, Formal analysis, Investigation, Writing – original draft. **Sema Sırma Ekmekci:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision. **Suzan Çınar:** Software, Visualization, Resources, Formal analysis. **Günnur Deniz:** Software, Resources, Writing – review & editing. **Neslihan Abacı:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Bray, J.E., Marsden, B.D., Oppermann, U., 2009. The human short-chain dehydrogenase/reductase (SDR) superfamily: a bioinformatics summary. Chem. Biol. Interact. 178 (1–3), 99–109. https://doi.org/10.1016/j.cbi.2008.10.058.

Chao, C.C., 2015. Mechanisms of p53 degradation. Clin. Chim. Acta 438, 139–147. https://doi.org/10.1016/j.cca.2014.08.015.

Crean, D., Felice, L., Taylor, C.T., Rabb, H., Jennings, P., Leonard, M.O., 2012. Glucose reintroduction triggers the activation of Nrf2 during experimental ischemia reperfusion. Mol. Cell. Biochem. 366 (1–2), 231–238. https://doi.org/10.1007/s11010-012-1300-4.

Deisenroth, C., Thorner, A.R., Enomoto, T., Perou, C.M., Zhang, Y., 2010. Mitochondrial Hep27 is a c-Myb target gene that inhibits Mdm2 and stabilizes p53. Mol. Cell. Biol. 30 (16), 3981–3993. https://doi.org/10.1128/MCB.01284-09.

Fang, L., Cheng, Q., Liu, W., Zhang, J., Ge, Y., Zhang, Q., Li, L., Liu, J., Zheng, J., 2016. Selective effects of a fiber chimeric conditionally replicative adenovirus armed with hep27 gene on renal cancer cell. Cancer Biol. Ther. 17 (6), 664–673. https://doi.org/10.1080/15384047.2016.1190485.

Gabrielli, F., Tofanelli, S., 2012. Molecular and functional evolution of human DHRS2 and DHRS4 duplicated genes. Gene 511 (2), 461–469. https://doi.org/10.1016/j.gene.2012.09.013.

Gabrielli, F., Donadel, G., Bensi, G., Heguy, A., Melli, M., 1995. A nuclear protein, synthesized in growth-arrested human hepatoblastoma cells, is a novel member of the short-chain alcohol dehydrogenase family. Eur. J. Biochem. 232 (2), 473–477. https://doi.org/10.1111/j.1432-1033.1995.473zz.x.

Geng, Q.Q., Dong, D.F., Chen, N.Z., Wu, Y.Y., Li, E.X., Wang, J., Wang, S.M., 2013. Induction of p53 expression and apoptosis by a recombinant dual-target MDM2/MDMX inhibitory protein in wild-type p53 breast cancer cells. Int. J. Oncol. 43 (6), 1935–1942. https://doi.org/10.3892/ijo.2013.2138.

Han, Y., Song, C., Wang, J., Tang, H., Peng, Z., Lu, S., 2018. HOXA13 contributes to gastric carcinogenesis through DHRS2 interacting with MDM2 and confers 5-FU resistance by a p53-dependent pathway. Mol. Carcinog. 57 (6), 722–734. https://doi.org/10.1002/mc.22793.

Han, Y., Wang, Z., Sun, S., Zhang, Z., Liu, J., Jin, X., Wu, P., Ji, T., Ding, W., Wang, B., Gao, Q., 2020. Decreased DHRS2 expression is associated with HDACi resistance and poor prognosis in ovarian cancer. Epigenetics 15 (1–2), 122–133. https://doi.org/10.1080/15592294.2019.1656155.

Heinz, S., Krause, S.W., Gabrielli, F., Wagner, H.M., Andreesen, R., Rehli, M., 2002. Genomic organization of the human gene HEP27: alternative promoter usage in HepG2 cells and monocyte-derived dendritic cells. Genomics 79 (4), 608–615. https://doi.org/10.1006/geno.2002.6743.

Honda, R., Tanaka, H., Yasuda, H., 1997. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS Lett. 420 (1), 25–27. https://doi.org/10.1016/s0014-5793(97)01480-4.

Lei, W., Rushton, J.J., Davis, L.M., Liu, F., Ness, S.A., 2004. Positive and negative determinants of target gene specificity in myb transcription factors. J. Biol. Chem. 279 (28), 29519–29527. https://doi.org/10.1074/jbc.M403133200.

Li, J.M., Jiang, G.M., Zhao, L., Yang, F., Yuan, W.Q., Wang, H., Luo, Y.Q., 2019. Dehydrogenase/reductase SDR family member 2 silencing sensitizes an oxaliplatin-resistant cell line to Oxaliplatin by inhibiting excision repair cross-complementing group 1 protein expression. Oncol. Rep. 42 (5), 1725–1734. https://doi.org/10.3892/or.2019.7291.

Luo, X., Li, N., Zhao, X., Liao, C., Ye, R., Cheng, C., Xu, Z., Quan, J., Liu, J., Cao, Y., 2019. DHRS2 mediates cell growth inhibition induced by Trichothecin in nasopharyngeal carcinoma. J. Exp. Clin. Cancer Res. 38 (1), 300. https://doi.org/10.1186/s13046-019-1301-1.

Monge, M., Colas, E., Doll, A., Gil-Moreno, A., Castellvi, J., Diaz, B., Gonzalez, M., Lopez-Lopez, R., Xercavins, J., Carreras, R., Alameda, F., Canals, F., Gabrielli, F., Reventos, J., Abal, M., 2009. Proteomic approach to ETV5 during endometrial carcinoma invasion reveals a link to oxidative stress. Carcinogenesis 30 (8), 1288–1297. https://doi.org/10.1093/carcin/bgp119.

Oppermann, U.C., Filling, C., Jörnvall, H., 2001. Forms and functions of human SDR enzymes. Chem. Biol. Interact. 130–132 (1–3), 699–705. https://doi.org/10.1016/s0009-2797(00)00301-x.

Pei, D., Zhang, Y., Zheng, J., 2012. Regulation of p53: a collaboration between Mdm2 and Mdmx. Oncotarget 3 (3), 228–235. https://doi.org/10.18632/oncotarget.443.

Pellegrini, S., Censini, S., Guidotti, S., Iacopetti, P., Rocchi, M., Bianchi, M., Covacci, A., Gabrielli, F., 2002. A human short-chain dehydrogenase/reductase gene: structure, chromosomal localization, tissue expression and subcellular localization of its product. Biochim. Biophys. Acta 1574 (3), 215–222. https://doi.org/10.1016/s0167-4781(01)00323-2.

Shafqat, N., Shafqat, J., Eissner, G., Marschall, H.U., Tryggvason, K., Eriksson, U., Gabrielli, F., Lardy, H., Jörnvall, H., Oppermann, U., 2006. Hep27, a member of the short-chain dehydrogenase/reductase family, is an NADPH-dependent dicarbonyl reductase expressed in vascular endothelial tissue. Cell. Mol. Life Sci. 63 (10), 1205–1213. https://doi.org/10.1007/s00018-006-6013-y.

Wang, J., Scholtens, D., Holko, M., Ivancic, D., Lee, O., Hu, H., Chatterton Jr., R.T., Sullivan, M.E., Hansen, N., Bethke, K., Zalles, C.M., Khan, S.A., 2013. Lipid metabolism genes in contralateral unaffected breast and estrogen receptor status of breast cancer. Cancer Prev. Res. (Phila.) 6 (4), 321–330. https://doi.org/10.1158/1940-6207.CAPR-12-0304.

Yuan, X., Sun, Y., Cheng, Q., Hu, K., Ye, J., Zhao, Y., Wu, J., Shao, X., Fang, L., Ding, Y., Sun, X., Shi, X., Xue, B., 2020. Proteomic analysis to identify differentially expressed proteins between subjects with metabolic healthy obesity and non-alcoholic fatty liver disease. J. Proteome 221, 103683. https://doi.org/10.1016/j.jprot.2020.103683.

Zhou, Y., Wang, L., Ban, X., Zeng, T., Zhu, Y., Li, M., Guan, X.Y., Li, Y., 2018. DHRS2 inhibits cell growth and motility in esophageal squamous cell carcinoma. Oncogene 37 (8), 1086–1094. https://doi.org/10.1038/onc.2017.383.

Zhou, Y., Fu, X., Zheng, Z., Ren, Y., Zheng, Z., Zhang, B., Yuan, M., Duan, J., Li, M., Hong, T., Lu, G., Zhou, D., 2020. Identification of gene co-expression modules and hub genes associated with the invasiveness of pituitary adenoma. Endocrine 68 (2), 377–389. https://doi.org/10.1007/s12020-020-02316-2.