In Silico Dissection of the Drug Sensitivity of Mesothelioma Cell Lines

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Abstract: Malignant mesothelioma is an extremely aggressive cancer of the mesothelial cells. Asbestos exposure and genetic predisposition are the two most well-established risk factors for mesothelioma occurrence. It has a high mortality rate with poor prognosis and high chemotherapeutic resistance via unknown mechanisms. In this study, we used in silico approach for studying the drug sensitivity response of 21 mesothelioma cell lines from Genomics of Drug Sensitivity in Cancer' (GDSC) database. We observed that only three cell lines displayed sensitivity to various drugs. Among these three cell lines, two mesothelioma cell lines displayed some commonalities in their drug sensitivities as well as their mutation profiles including, mutation spectrums, the flanking regions of the mutated base, and their respective heatmaps.

1   INTRODUCTION

Malignant mesothelioma (MM) is an aggressive cancer of the mesothelial cells with poor prognosis (Zalcman et al. 2016) and ahigh mortality rate (Carbone et al. 2012).The predicted number of incidence is alarming; over 20 million people in the US alone are on the verge of developing MM due to asbestos exposure(Carbone et al. 2012) and the global MM incidence and the resulting mortality rates may be even higher for the developing nations which happens to use significantly higher amount of asbestos than the developed countries (Carbone et al. 2019).There have been several attempts to develop drugs for MM using doxorubicin, cyclophosphamide, cisplatin, carboplatin, gemcitabine, pemetrexed, ethyl pyruvate, and tremelimumab(Samson et al. 1987; Chahinian et al. 1993; Byrne et al. 1999; White et al. 2000; Kindler et al. 2001; Hughes et al. 2002; Calabrò et al. 2013; Pellegrini et al. 2017)in the past. However, the majority of patients die within 24 months of diagnosis often due to   high chemotherapeutic resistance via unknown mechanisms(Cortes-Dericks et al. 2010; Mujoomdar et al. 2010; Tajima et al. 2010; Cregan et al. 2016). The effective treatment of mesothelioma requires a multidimensional approach such as finding novel targets and finding suitable biomarkers for the resistant and sensitive cell lines. In this study, we used in silico approach to dissect the drug sensitivity of MM cell lines.

1.1 Primary Mesothelial Cell Lines

Primary cultures of mesothelial cells have been established from rats, rabbits, mice, and humans. Mesothelial cell lines provide several advantages for experimental studies: they provide a large number of cells isolated from a single donor, cell lines can be isolated from genetically engineered mice, and primary cell lines limit the number of animals required for experiments. However, cell lines have several disadvantages: variability among donors, variability in culture conditions in different laboratories, potential phenotypic and genetic instability, and a limited life span in vitro. Some of these disadvantages can be overcome by quality control procedures.

For example, cell lines should not be passaged indefinitely; frozen stocks should be maintained and thawed at regular intervals to prevent phenotypic and genetic instability. As in all cell culture models, precautions are required to prevent cross-contamination and contamination with bacteria or viruses. DNA profiles could be useful to identify cell lines; for example Manning et al established initial

genetic profiles for their panel of human malignant mesothelioma cell lines. All cultures should be screened for *Mycoplasma* and other pathogens (Masters, et al. 2000).

Technical details regarding primary human mesothelial cell cultures have been summarized by

Versnel et al (Versnel,et al. 1994) and Gerwin (Gerwin, et al. 1994). Briefly, primary human mesothelial cells require enriched culture media supplemented with 10% to 20% fetal bovine serum, exogenous growth factors [usually epidermal growth factor (EGF)], insulin, transferrin, and hydrocortisone. Rabbit, mouse, and rat primary mesothelial cells require similar growth conditions, with the important exception that growth of rat pleural mesothelial cells is inhibited by EGF. As reviewed by Walker et al , there are additional differences in expression of growth factors and their receptors between human and rat mesothelial cells. Differences in growth factor responses have been described in primary human mesothelial cell cultures derived from different donors (Lechners, et al. 1989).

Mesothelial cell cultures have been characterized by morphology, electronmicroscopy, immunocytochemistry, and cytogenetic). Although mesothelial cells can form monolayers with epithelial morphology, this growth pattern can be altered in vitro as described below.

At the ultrastructural level, mesothelial cells typically show surface microvilli, abundant mitochondria, extensive rough endoplasmic reticulum, perinuclear intermediate filaments, desmosomes, and tight junctions. Immuno cytochemistry is useful to confirm expression of markers specific for mesothelial cells, especially coexpression of intermediate filaments, keratin, and vimentin (Mackay, et.al. 1987) and expression of the Wilms' tumor suppressor gene, *WT1* (Walker, et al. 1994). These markers are also useful for the immunohistochemical diagnosis of human malignant mesotheliomas (Zeng, et al, Ordozen, et al. 2002). Cytogenetic studies of human mesothelial cell lines reveal a normal karyotype that may acquire abnormalities after several passages (Versnel, et al. 1994). One primary murine mesothelial cell line has been reported that spontaneously acquired a point mutation in exon 5 of the *p53* tumor suppressor gene. This mutation increased growth rate in vitro; however, it did not confer tumorigenicity (Cistulli, et al. 1992).

Primary cell lines provide a valuable model to study the cell biology and differentiation of normal mesothelial cells. Primary cultures have also been used to investigate the toxicologic effects of asbestos and man-made mineral fibers (Lechner, et al. 1991).

The mesothelium is derivedembryologically from the mesoderm. At approximately embryonic day 7.5 in the mouse, epithelial cells undergo mesenchymal differentiation to form the mesoderm cell layer. This morphologic differentiation is governed by transcription factors *snail* and *slug* that modulate expression of cadherins and cytoskeletal proteins characteristic of mature mesothelial cells (Carver, et al. 2001). In response to mechanical injury, peritoneal dialysis, or chronic inflammation, mesothelial cells also revert from an epithelial to a mesenchymal phe- notype. This transdifferentiation is termed the epithelial-mesenchymal transition and has been investigated in primary cultures of human mesothelial cells isolated from reactive peritoneal effusions or dialysis effluent. In these pathologic conditions, human mesothelial cells detach from the mesothelial monolayer and survive in suspension.

When these reactive mesothelial cells are placed in monolayer culture, they express epithelial or mesenchymal phenotypes (Carver, at al. 2001) characterized the expression of cytoskeletal proteins including actin, vimentin, and several cytokeratins by mesothelial cells isolated from ascitic fluid. Modulation of the epithelial phenotype in vitro depended on culture conditions: serum, EGF, and hydrocortisone induced a mesenchymal phenotype, while supplementation with retinoic acid induced an epithelial phenotype. The epithelial‐ mesenchymal transition of reactive human mesothelial cells in vitro is characterized by reduced expression of some cell surface proteoglycans (syndecan-4, glypican-1), the *WT1* tumor suppressor gene, and decreased expression of E cadherin in parellel with expression of the transcription factor *snail*. Transdifferentiation of omental mesothelial cells in vitro was also induced by mechanical wounding of mesothelial monolayers or by exposure to the inflammatory mediators, transforming growth factor-b1 (TGF-b1) or interleukin-1b (IL-1b) (Carver, at al. 2001).

Mesothelial cells are sensitive target for transformation by asbestos fibers. The biologic basis for this increased sensitivity is unknown. Studies conducted with cell culture models have provided evidence that the iron-catalyzed generation of reactive oxygen species is a plausible mechanism for asbestos carcinogenicity. Reactive oxygen species have been implicated in asbestos-induced apoptosis, chromosomal damage, oxidative DNA damage, and DNA strand breaks (Ollikainen, et al. 1996) in human and rat pleural mesothelial cells. Variations in antioxidant defense mechanisms have been hypothesized to contribute to pulmonary disease induced by fibers and particulates (Driscoll, et al. 2002). The antioxidant defense pathways of primary rat pleural mesothelial cells have been characterized in detail; these cultures have low catalase activity

and depend primarily on the glutathione pathway for protection against oxidant stress (Kinnula, et al. 1992). These mechanistic studies suggest that mesothelial cells are highly susceptible to DNA and chromosomal damage in response to asbestos exposure. Mesothelial cells with asbestos-induced DNA damage that escape apoptosis may be precursors for the development of malignant mesothelioma (Broaddus, et al. 1996).

2 METHOD

2.1 Mesothelioma Cell Lines

The list of mesothelioma cell lines and their respective COSMIC ids were obtained from the Genomics of Drug Sensitivity in Cancer' (GDSC) database.

2.2 Datasets for Drug Response

The GDSC datasets are generated as a result of various projects and are categorized into two datasets, GDSC1 and GDSC2. The original dataset of GDSC was expanded in the form of GDSC1 by integrating heterogeneous molecular data of 11,289 tumors and 1,001 cell lines and measuring the response of 1,001 cancer cell lines to 265 anti-cancer drugs (Iorio et al. 2016) jointly by Wellcome Sanger Institute and Massachusetts General Hospital between 2009 and 2015. In contrast, the GDSC2 dataset was generated by Wellcome Sanger Institute using the improved methods for screening and assays. Considering that GDSC2 a more reliable dataset, all our data are obtained from it and not from GDSC1.

2.3 Chemical Structures of Drugs

The chemical structures of the sensitive drugs were obtained from the Inxight: Drugs portal of the National Center for Advancing Translational Sciences (NCATS).

2.4 Mutation Spectrums

The mutations spectrum of the cell lines were obtained from the 'catalogue of somatic mutations in cancer (COSMIC) portal. The mutation spectrum plot displays all the substitution nucleotide base pair changes on the Y-axis and the frequency on X-axis. It shows the frequency of six substitution classes (C:G>A:T, C:G>G:C, C:G>T:A, T:A>A:T,

T:A>C:G & T:A>G:C) and indels (which is used for insertion or deletion of bases in the genome).

2.5 Flanking Regions of Mutated Bases

The flanking sequence for all mutations referenced to the pyrimidine base (T>X, T>G, T>C, T>A: C>X, C>T, C>G, C>A) for each cell lines were obtained from the COSMIC portal available at Sanger web server. It displays the mutated base at position 0 together with the frequency for the 10 bases at the upstream and downstream of the mutated base.

2.6 Genomic Heatmaps of Mesothelioma Cell Lines

The genomic heatmaps from the cell line projects were obtained for NCI-H2795, NCI-H513, and MSTO-211H cell lines. These heatmaps were constructed from counts of each mutation-type at each mutation context corrected for the frequency of each trinucleotide in the coding region of the reference genome. The plot shows the log-transformed values of these ratios. The 5′ base to each mutated base is shown on the vertical axis and 3′ base on the horizontal axis.

3 RESULT

3.1 Mesothelioma Cell Lines in GDSC

The 'Genomics of Drug Sensitivity in Cancer' (GDSC) database allows access to the drug sensitivity datasets on a large number of 1001 cell lines of which 990 cells lines have drug response data available (Supplementary materials S1). Among 1001 cell lines, 21 belong to mesothelioma cancer type. These cell lines include NCI-H2369, NCI-H2373, NCI-H2461, NCI-H2591, NCI-H2595, NCI-H2722, NCI-H2731, NCI-H2795, NCI-H2803, NCI-H2804, NCI-H2810, NCI-H2818, NCI-H2869, NCI-H290, NCI-H513, NCI-IST-MES1, NCI-MPP-89, NCI-MSTO-211H, NCI-H2052, NCI-H2452, and NCI-H28.

3.2 Drugs Sensitivity Response of Mesothelioma Cell Lines and Their Target Pathways

In the GDSC2 dataset majority of mesothelioma cell lines (17 out of 21) including,

NCI-H2369, NCI-H2373, NCI-H2461, NCI-H2591, NCI-H2595, NCI-H2722, NCI-H2731, NCI-H2803, NCI-H2804, NCI-H2810, NCI-H2818, NCI-H2869, NCI-H290,NCI-IST-MES1, NCI-MPP-89, NCI-H2052, NCI-H2452, and NCI-H28 exhibited no sensitivity to any drugs. In contrastonly 3 of the mesothelioma cell lines including NCI-H2795,NCI-H513, and MSTO-211Hexhibited sensitivity to different drugs. The cell line NCI-H2795 was sensitive to three different drugs PD173074, AZD4547, and Cediranib (Table 1, Fig. 1).
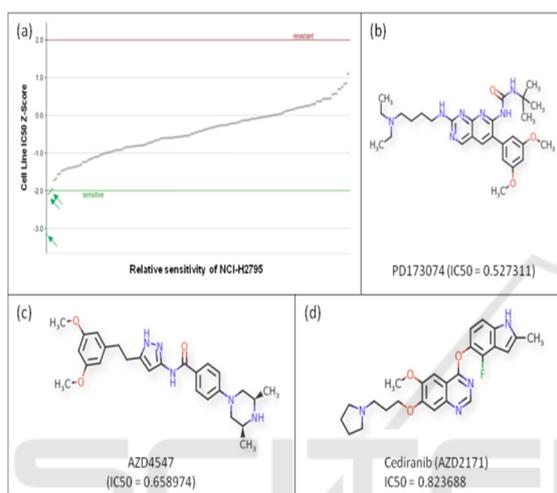

Figure 1: Drug sensitivity of mesothelioma cell lines NCI-H2795 (a). The cell line is sensitive to PD173074 (b), AZD4547, and (c) Cediranib.

Whereas, the other two cell lines NCI-H513, and MSTO-211H are sensitive to Acetalax, and PD173074, respectively (Table 1, Fig. 2).

Table 1: Drug sensitivity of mesothelioma cell lines

S.No.	Cell Lines	Sensitivity to Drugs	Targets	IC50
1	NCI-H2369	-	-	-
2	NCI-H2373	-	-	-
3	NCI-H2461	-	-	-
4	NCI-H2591	-	-	-
5	NCI-H2595	-	-	-
6	NCI-H2722	-	-	-
7	NCI-H2731	-	-	-
8	NCI-H2795	PD173074	FGFR1, FGFR3	0.527311
		AZD4547	FGFR1, FGFR2, FGFR3	0.658974
		Cediranib	VEGFR, FLT1, FLT2, FLT3, FLT4, KIT, PDGFRB	0.823688
9	NCI-H2803	-	-	-
10	NCI-H2804	-	-	-
11	NCI-H2810	-	-	-
12	NCI-H2818	-	-	-
13	NCI-H2869	-	-	-
14	NCI-H290	-	-	-
15	NCI-H513	Acetalax	-	1.084383
16	IST-MES1	-	-	-
17	MPP-89	-	-	-
18	MSTO-211H	PD173074	FGFR1, FGFR3	2.17617
19	NCI-H2052	-	-	-
20	NCI-H2452	-	-	-
21	NCI-H28	-	-	-

Thecell line NCI-H2795 was found to be sensitive for three different drugsPD173074, AZD4547, and Cediranib. The PD173074 is inhibitory to FGFR1, and FGFR3; AZD4547 inhibits FGFR1, FGFR2, and FGFR3. The interesting commonality between the two drugs is that both inhibitfibroblast growth factor receptors (FGFRs) thereby inhibiting thesignal transduction pathways, and, so, the inhibition of tumor cell proliferation and tumor cell death. Up-regulation of FGFR, which is a tyrosine kinase receptor, has been reported in many tumors, and the sensitivity of NCI-H2795 to the drugs PD173074 and AZD4547 suggests theover-expression of FGFRs as the major driving force for mesothelioma. Similarly, drug cediranib is a potent inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinases. Considering the targets of all these three drugs for which NCI-H2795 is sensitive, it is conceivable that the tyrosine kinase receptors such as FGFRs and VEGF are over-expressed in mesothelioma and are essential to tumor cellular proliferation, differentiation and survival.Like NCI-H2795, the cell line MSTO-211His also sensitive to the PD173074, the FGFRs inhibitor. In contrast, the NCI-H513cell line is sensitive to Acetalax, which is a laxative and its specific target is largely unknown. However,

Acetalax has been shown to trigger a cell starvation response leading to autophagy, mitochondrial dysfunction, and autocrine TNFα-mediated apoptosis(Morrison et al. 2013).
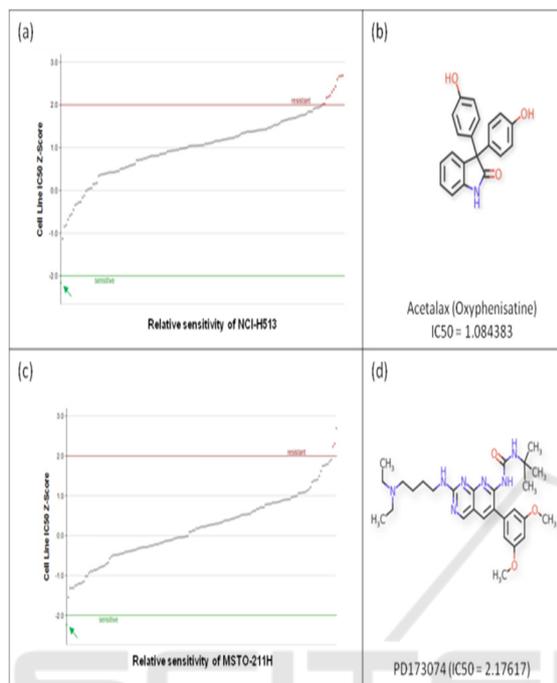

Figure 2: Drug sensitivity of mesothelioma cell lines NCI-H513 and MSTO-211H. (a-b) NCI-H513 is sensitive to Acetalax. (c-d) MSTO-211H is sensitive to PD173074.

3.2 Mutation Spectrum of Sensitive Cell Lines

The mutation spectrum of cell lines NCI-H2795 and MSTO-211H displays some degree of similarity. In both cases, the frequency of C:G>T:A substitution is 630 and 871, respectively, which are highest among all the different substitution classes. Additionally, the class of the second most frequent substitution in both the cell line is also the same; the T:A>C:G substitution in NCI-H2795 and MSTO-211H is 375 and 415, respectively (Fig. 3a and b). In contrast to NCI-H2795 and MSTO-211H, the class of the most frequent substitution in NCI-H513 is C:G>A:T followed by C:G>T:A, though the number of total mutations in each substitution class in NCI-H513 is significantly higher compared to the other two cell lines (Fig. 3c).
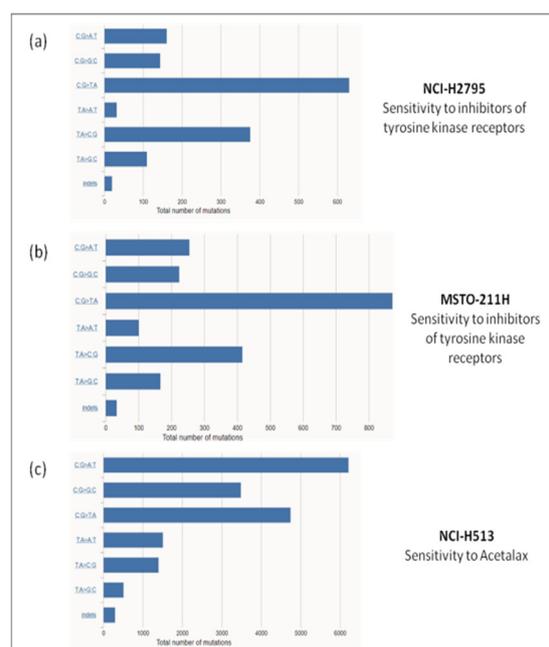

Figure 3: Mutation spectrum of mesothelioma cell lines NCI-H2795, MSTO-211H, and NCI- H513.

3.3 Flanking Regions of Mutated Base

Apart from the mutation spectrum, the flanking sequence for all mutations referenced to the pyrimidine base (T>X, T>G, T>C, T>A: C>X, C>T, C>G, C>A) for each cell lines were also analyzed to test if there is any similarity in the cell lines NCI-H2795 and MSTO-211H. Interestingly, we observed that thenucleotide frequency of ten bases upstream and downstream of the T>X and C>X mutations were maximum for T>C and C>T, respectively for both NCI-H2795 and MSTO-211H (Fig. 4). As conceivable, the nucleotide frequency of ten bases upstream and downstream of the T>X and C>X mutations were maximum for T>A and C>A for the cell line NCI-H513 (Fig. 5).
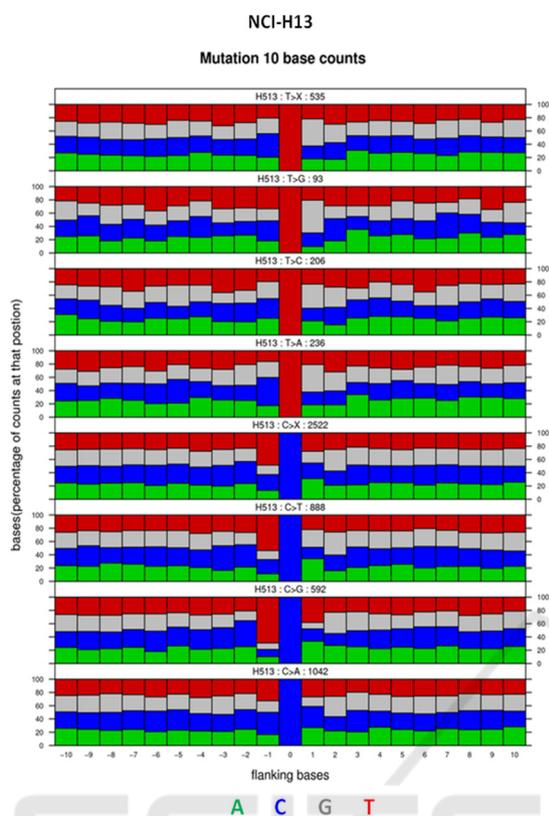
Figure 5: Extended sequence context diagram of mesothelioma cell line NCI-H513.

The plots shows 21bp sequence context, combining data from all mutations in a single sample. The nucleotide frequencies of ten bases upstream and downstream of the mutated base are shown normalised to the frequency across the coding region of the genome.

3.4 Genomic Heatmaps of Mesothelioma Cell Lines

The deamination of cytosine in a CpG dinucleotide context (emphasized by 3, 7, 11, and 15 of the mutation class C>T) is one of the common features of the genomic heatmaps of all the three mesothelioma cell lines, NCI-H2795, MSTO-211H, and NCI-H513 (Fig. 6).
The genomic heatmaps of NCI-H2795 and MSTO-211H were observed to be quite similar to each other (Fig. 6a and b). The XpCpA and XpCpT (emphasized by 1, 5, 4 and 12 in Fig. 6a and by 3, 9, and 14 in Fig. 6b) are rarely mutated in NCI-H2795 and MSTO-211H (Fig. 6a and b). In the mutation class C>A, the TpCpG (emphasized by 15 of the

mutation class C>A in Fig. 6a) of NCI-H2795 and the GpCpG (emphasized by 11 of the mutation class C>A in Fig. 6b) of MSTO-211H are frequently mutated. Additionally, in the mutation class C>G, the triplet ApCpG (emphasized by 3 of the mutation class C>G in Fig. 6a) of NCI-H2795 and the TpCpG (emphasized by 15 of the mutation class C>G) in MSTO-211H have frequently mutated bases. Moreover, the mutation classes T>A and T>G are rarely mutated in both NCI-H2795 and MSTO-211H.In contrast to NCI-H2795 and MSTO-211H, the cell line NCI-H513 exhibited a high frequency of mutations in C>A and C>G classes (Fig. 6c).
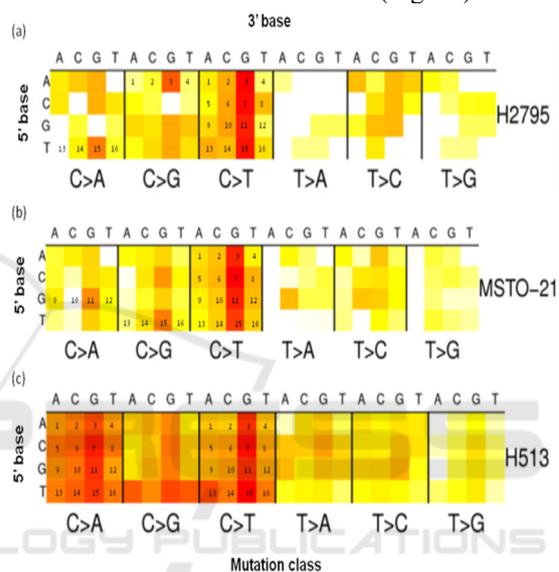

Figure 6: Genomic heatmap of mesothelioma cell lines NCI-H2795, MSTO-211H, and NCI-H513.

The heatmap shows the frequency of mutations for all possible triplet bases normalised against the frequency across the coding genome. These triplets are composed of the mutated base together with the 5' and 3' bases. There are 96 possible triplets, 16 for each mutation class (C>A, C>G, C>T, T>A, T>C, and T>G).

5 CONCLUSION

A vast majority of mesothelioma cell lines in the GDSC database did not display sensitivity to any of the drugs tested so far. Drug response data shows that only three cell lines including NCI-H2795, NCI-H513, and MSTO-211H exhibitedsensitivity to different drugs. The NCI-H2795 was sensitive to PD173074, AZD4547, and cediranib, while MSTO-

211H and NCI-H513 cell lines are sensitive to PD173074, and acetalax, respectively.

Interestingly, the targets of the drugsPD173074, AZD4547, and cediranib are tyrosine kinase receptors such as FGFRs and VEGF suggesting that the tyrosine kinase receptors in the two mesothelioma cell lines, NCI-H2795 and MSTO-211H, are essential to tumor cellular proliferation, differentiation, and survival. Unlike NCI-H2795 and MSTO-211H, the NCI-H513 cell line is sensitive to Acetalax (and resistant to other drugs), which is a laxative and its specific target is largely unknown.

It is conceivable that the genomic mutation profile of the cell lines, NCI-H2795 and MSTO-211H, which display similarity in their response to drugs, is likely similar. Therefore, we also looked into the mutation spectrum, flanking regions of the mutated bases, and the heatmaps of the substitution mutations of these cell lines. As expected these displayed a very similar mutation profile, which is strikingly different that of the NCI-H513. This information along with any future study involving the study of the transcriptomic profile of resistant and sensitive cell lines could provide us with suitable biomarkers for drug sensitivity response.

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