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Bioinformatics Analysis of essential genes in *NF1* and *CDKN2A* null cancer cell lines

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Abstract

Neurofibromatosis type 1 (NF1) is a common cancer predisposition syndrome characterized by a germline loss of function mutation in the *NF1* tumor suppressor gene. The heterozygous loss of one copy of this gene drives the clinical condition, but the occasional biallelic loss of *NF1* in the Schwann cell lineage causes the formation of tumors called neurofibromas in almost all patients. The further loss of function of a second tumor suppressor, *CDKN2A*, is associated with transformation into an atypical neurofibroma, which can then transform into an aggressive malignant peripheral nerve sheath tumor (MPNSTs). Cells that lose certain genes can become more reliant on other pathways, which can be targeted for treatments in a phenomenon called synthetic lethality. Treatments of MPNST and precursor atypical neurofibromas lacking *NF1* and *CDKN2A* have not yet been identified. Using the Cancer Dependency Map Project and Ingenuity Pathway Analysis, canonical and upstream pathway associations were identified from genes selectively essential in cell lines containing both *NF1* and *CDKN2A* null mutations. Genes involved in estrogen receptor signaling were enriched in the list of essential genes in *NF1* and *CDKN2A* co-deleted cells, with IGF-1, PGR, CDK8, ADCY1, and PKCε identified as potential drug targets from the pathway. The *SMARCA4* upstream regulatory pathway was also found to be significantly enriched, which has previously been implicated as a synthetic lethal target for certain cancers. These targets can be used for identifying novel therapies targeting MPNST pre-malignant precursors to prevent malignant transformation of neurofibromas and improve health outcomes for NF1 patients

Background

Neurofibromatosis Type 1 clinical manifestations

Neurofibromatosis Type 1 (NF1) is a common genetic cancer predisposition syndrome estimated to affect over 2 million people worldwide (Gutmann et al., 2017). This autosomal dominant genetic disorder is caused by the germline loss of a functional copy of the *NF1* tumor suppressor gene. The loss of this *NF1* tumor suppressor occurs in 1 in 3000 births, with half of NF1 cases being spontaneous or de novo and half are inherited from a parent. NF1 affects multiple organ systems and results in a life expectancy of 15 years lower than average, primarily due to malignancies (Rasmussen and Friedman, 2000). Patients present with a wide array of symptoms, including flat light brown markings, freckling in armpits, hamartomas on

the iris, scoliosis, and benign tumors forming on peripheral nerves called neurofibromas (Young et al., 2002). This disease's clinical manifestations are highly variable as the different phenotypes of this disease are variably penetrant (Sabbagh et al., 2009).

Neurofibromas are a hallmark of this genetic condition and occur in over 99% of NF1 patients, with potential serious health consequences such as transformation into aggressive cancers (Le and Bedocs, 2020). Preventing the malignant transformation of neurofibromas by targeting precursors would improve health outcomes of NF1 patients (Staedtke et al., 2017). These tumors originate from the Schwann cell lineage in several different manifestations (Zhu et al., 2002). Cutaneous neurofibromas arise in the skin and accumulate over a patient's lifetime, but generally do not grow larger than three centimeters and present a very low risk of transformation into malignancy (Ferner and Gutmann, 2013). Plexiform neurofibromas are a more serious category of neoplasm that grow and proliferate on internal nerves in 20-50% of NF1 patients, with a significant risk of malignant transformation (Korf, 1999). Neurofibromas that undergo transformation become malignant peripheral nerve sheath tumors (MPNSTs), which have a 5-year survival rate of 21% (Evans et al., 2002). MPNST's are rare in the general population, with a lifetime risk of 0.001%, but represent a serious risk for NF1 patients, that have an 8-13% lifetime risk of this type of sarcoma (Ferrari et al., 2007).

Molecular basis of NF1

The genetic basis of the NF1 syndrome is the germline loss of one copy of the *NF1* gene which codes for the tumor-suppressing protein neurofibromin (Le and Bedocs, 2020). Neurofibromin functions as a suppressor of Ras, a small GTP binding protein that hydrolyzes GTP to GDP over time (Cichowski and Jacks, 2001). When Ras binds GTP, it switches into an active conformation and turns on the MAPK and PI3K/AKT proliferative signaling pathways (Gimple and Wang, 2019). Overactivation of these pathways is a known driver of cancer (Thompson et al., 2015). Neurofibromin promotes the hydrolyzation of bound GTP to GDP in Ras to inactivate the molecule and turn off proliferative signaling pathways, suppressing growth (Cichowski and Jacks, 2001). Though lower neurofibromin levels in NF1 patients are the cause of the wide range of symptoms seen, the heterozygous genotype is not enough to cause tumor formation (Carroll and Ratner, 2008). The primary event needed for the creation of a neurofibroma is the loss of the second copy of the *NF1* gene, a theory known as the second hit hypothesis (Carroll and Ratner, 2008). This hypothesis postulates the complete loss of neurofibromin significantly reduces inhibition of Ras and causes significant overactivation of downstream proliferative pathways (Jouhilahti et al., 2011).

Following the initial biallelic loss of *NF1*, a series of genetic transformations are required for further tumor progression (Rhodes et al., 2019). Certain neurofibromas progress first to atypical neurofibromatous neoplasms of uncertain biological potential (ANNUBP) and then into MPNSTs (Rhodes et al., 2019). The loss of the tumor suppressor gene *CDKN2A* has been found to promote the transformation of neurofibromas into ANNUBP's (Rhodes et al., 2019). This gene codes for a tumor suppressor cyclin-dependent kinase inhibitor 2A (CDKN2A) that has several

mRNA variants, one of which encodes the $p14^{Arf}$ protein which stabilizes the critical tumor suppressor protein p^{53}, which is mutated in 50% of cancers (Ozaki and Nakagawara, 2011; Zhao et al., 2016). *CDKN2A* also produces an mRNA transcript encoding the $p16^{INK4a}$ protein, a cyclin-dependent kinase inhibitor that slows cell division (Laud et al., 2006). Loss of *CDKN2A* can lead to unregulated cell division, and loss of this gene in mice was found to induce growth in *NF1* null Schwann Cells that mirrored human ANNUBP that progressed to MPNST's (Rhodes et al., 2019).

Synthetic lethality in cancer drug target discovery

Given the importance of the loss of *NF1* and *CDKN2A* in causing progression of normal Schwann cells into pre-malignant peripheral nerve sheath tumors, this project focused on identifying cancer cell lines with biallelic deletion mutations of these two genes and the discovery of genes that are only essential in the irregular cell lines. Essential genes are classified as indispensable for the growth of a regular cell, and loss of these functions leads to cell death (Riddle et al., 1997). However, cells often have mechanisms to maintain genetic robustness and can compensate for the loss of a particular gene with the another's function (Nijman, 2011). When these mechanisms fail a phenomenon called synthetic lethality occurs, where the suppression of a combination of two genes leads to lethality, even though individual supression is nonlethal (Gao and Lai, 2018). This project aimed to identify genes essential in only *NF1* and *CDKN2A* null cell lines and analyze signaling pathways common across the results.

Analyzing gene dependencies in the Cancer Dependency Map Project

The analysis of gene essentialities was done using the Cancer Dependency Map Project data (DepMap) (Dempster et al., 2019; Meyers et al., 2017). The DepMap project classifies gene essentiality across a wide range of cancer cell lines, with a goal of identifying targeted treatments for cancers (Dempster et al., 2019). DepMap uses both a CRISPR-Cas-9 knockout dataset as well as an RNA interference (RNAi) dataset. These datasets can have slightly different results due to differences in the molecular screening techniques used. In RNAi gene silencing, double-stranded RNA is taken up by the cell and processed by enzymes and proteins that destroy or suppress identical mRNA in the cell (Agrawal et al., 2003). The CRISPR-Cas-9 knockouts were created by using the RNA-guided DNA endonuclease Cas-9. Cas-9 is able to find its target sequence in genomic DNA using a synthetic single guide RNA (sgRNA), and proceeds to splice out the sequence of interest (Redman et al., 2016). This has some benefits over RNAi by having less off-target effects and higher efficiency at generating complete gene knockouts or loss of function mutations (Boettcher and McManus, 2015). However, RNAi can show more subtle knockdown effects compared to CRISPR-Cas-9 with an effect that is more similar to drug suppression rather than a complete loss of function (Boettcher and McManus, 2015).

In both the RNAi and CRISPR DepMap datasets, a screen of cancer cell lines was analyzed by knocking out various genes. The effect of these mutations on the cells was tabulated by a score called gene dependency (Shimada et al., 2019). This score is centered around 0 and represents the change in viability caused by the loss of a certain gene. Generally, a gene is considered essential if it has a dependency less than -0.5, which represents significant inhibited growth in the cell line if that

gene is knocked out (Joshua Dempster, 2020). To examine the gene dependencies in *NF1* and *CDKN2A* null cell lines, the DepMap RNAi and CRISPR-Cas-9 datasets were both analyzed in R Studio (Laurent Gatto and Theo Killian, 2020; RStudio Team, 2020; Shimada et al., 2019). The resulting essential genes were compared in Ingenuity Pathway Analysis (IPA), a program that identifies connections between genes (Krämer et al., 2014; QIAGEN Inc.).

Results

Tumors that become malignant in NF1 patients frequently undergo a complete loss of *NF1* and *CDKN2A*. To examine the various essential genes in cancer cells missing *NF1* and *CDKN2A*, the DepMap mutations dataset was filtered for cell lines containing deleterious mutations in *NF1* and *CDKN2A*. Eight cancer cell lines were identified with null mutations in both genes (Supplementary Table 1). The full CRISPR and RNAi gene dependency datasets from DepMap were then filtered for all data from only these cell lines of interest. To compare these gene dependencies against average gene dependencies, the CRISPR and RNAi datasets were analyzed to determine the average gene dependency for every gene in the database. The average gene dependencies were then subtracted from the dependencies of respective genes in the cell lines of interest, generating a list of genes sorted by the biggest difference in gene essentiality. All genes that were considered universally essential were eliminated (Joshua Dempster, 2020; Zhan and Boutros, 2016). The top 500 genes with the largest difference in gene dependencies were exported from both the RNAi and CRISPR data set (Supplementary Tables 3 and 4).

The gene dependency lists were analyzed in Ingenuity Pathway Analysis (IPA) to identify the most strongly associated canonical pathways (Figure 1). The RNAi gene dependencies show weaker associations due to the knockdown nature of the technology, but can be compared to the CRISPR dataset to show trends (Boettcher and McManus, 2015). This analysis was based on comparing Z-scores, known as an "Activation Score" in IPA. A positive Z-score corresponds to a pathway that were overrepresented in the gene dependency datasets, while negative Z-scores correspond to under-represented pathways. The pathways associated in both datasets showed stronger evidence of being truly associated with *CDKN2A* and *NF1* null mutations (Figure 2).

To further investigate the most strongly upregulated pathway from the CRISPR dataset, the estrogen receptor pathway associations were mapped (Figure 3). G-alpha was found to be significantly enriched in the essential genes of both the RNAi and CRISPR-Cas-9 datasets, with the CRISPR-Cas-9 knockout dataset finding more associated gene products present in the pathway. The gene products CDK8, IGF1, PGR, and PRKCE were implicated as potential anti-cancer targets with drugs inhibitors currently on the market (Supplementary Table 2). Upstream regulator analysis of the two datasets was performed, with a comparison of the upstream genes associated with both the CRISPR and RNAi dataset of selectively essential genes (Figure 4). A strong positive upregulation of SMARCA4 and a negative association of miR-124-3p was identified from the Z-score results. Thus, essential genes in *NF1* and *CDKN2A* co-deleted cancer cell lines are enriched for targets of the transcriptional activator SMARCA4

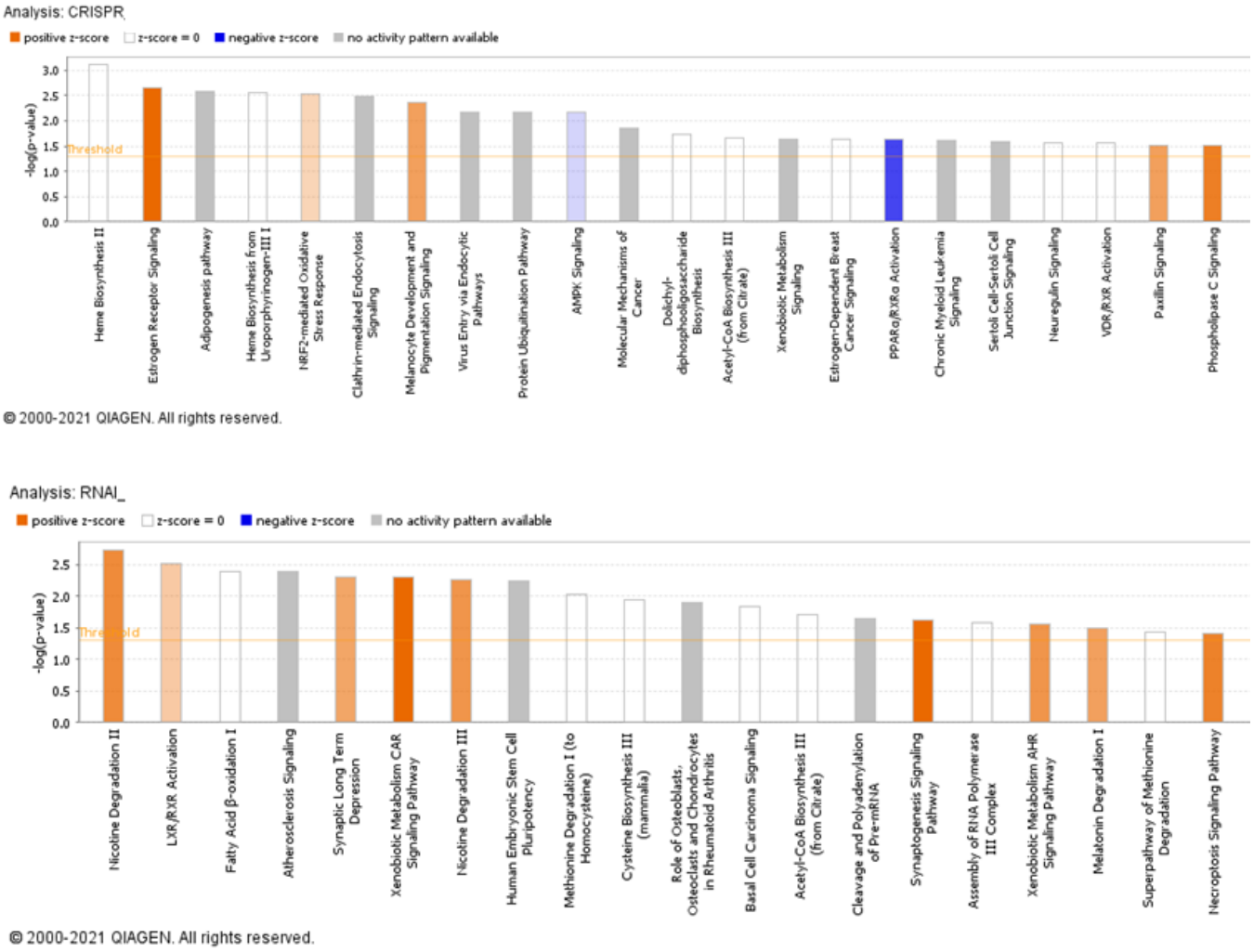

Figure 1 *Canonical Pathway associations of essential genes in NF1 and CDKN2A knockouts in both the CRISPR-Cas-9 and RNAi datasets.* Ingenuity Pathway Analysis of selectively essential genes in *NF1* and *CDKN2A* knockouts in CRISPR-Cas-9 dataset (top row) and RNAi datasets (bottom row). The threshold for statistical significance of $p < 0.05$ is shown by the orange line. The strongest positively associated pathway in the CRISPR dataset was Estrogen Receptor Signaling and Melanocyte development, while the RNAi dataset showed the strongest positively associated pathways to be Nicotine Degradation, LXR/RXR activation, Synaptic Long-Term Depression, and Xenobiotic Metabolism.

Discussion

Gene dependency analysis uncovered many significantly correlated pathways and upstream regulators related to NF1-associated tumor progression. The CRISPR-Cas-9 dataset technology has more effective targeting, suggesting that the pathways correlated to the biallelic null mutations of *CDKN2A* and *NF1* in the CRISPR dataset were more reliable than the RNAi dataset (Boettcher and McManus, 2015) The strongest positively associated signaling pathway in the CRISPR dataset was the estrogen receptor signaling pathway, and additional positive correlations were found in a comparative analysis of the RNAi dataset (Figure 1 and 2). This signaling pathway was more highly

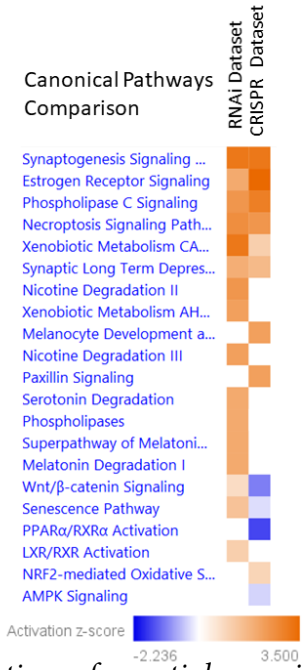

Figure 2 *Canonical Pathway associations of essential genes in NF1 and CDKN2A knockouts in both CRISPR-Cas-9 and RNAi datasets.* Ingenuity Pathway Analysis of selectively essential genes in *NF1* and *CDKN2A* knockouts in CRISPR-Cas-9 dataset (left column) and RNAi datasets (right column). The activation Z-score corresponds to if the pathway was over (orange) or under-represented (blue) by the gene dependencies of the two datasets. The most associated pathways in both datasets were synaptogenesis signaling, estrogen receptor signaling, necroptosis signaling, xenobiotic metabolism, and synaptic long-term depression signaling.

associated with the selectively essential genes by three standard deviations in the CRISPR and RNAi datasets compared to the average association in the datasets (Figure 2). Many proteins found in this signaling pathway are all known components in the NF1 condition, including Ras, a protein directly regulated by neurofibromin (Hannan et al., 2006). Previous work has shown that NF1 tumors often present with upregulated estrogen receptor signaling (Dischinger et al., 2018).

Potential gene product targets from the estrogen signaling pathway associated with selectively essential genes in NF1 and CDKN2A deficient cancer cell lines

The analysis revealed several specific gene targets that could potentially be targeted in this pathway including IGF-1, PGR, CDK8, PKCε, and ADCY1 (Supplementary Table 2). IGF-1 is known to be associated with NF1, and IGF-1R has been found to be upregulated in NF1-associated peripheral nerve sheath tumors (Friedrich et al., 2007). The IGF1R is already currently being studied as a potential therapeutic target for MPNSTs (Yang and Zhang, 2012). The progesterone receptor (PGR) is another target with a multitude of known drugs with an established association with NF1 (McLaughlin and Jacks, 2003). Increased progesterone receptor expression was found much more frequently than estrogen receptor and more often in plexiform neurofibromas (Geller et al., 2008). Targeting this receptor would allow for selective targeting of MPNST precursor cells, which could be tested using a wide variety of different drugs currently on the market that target PGR (Supplementary

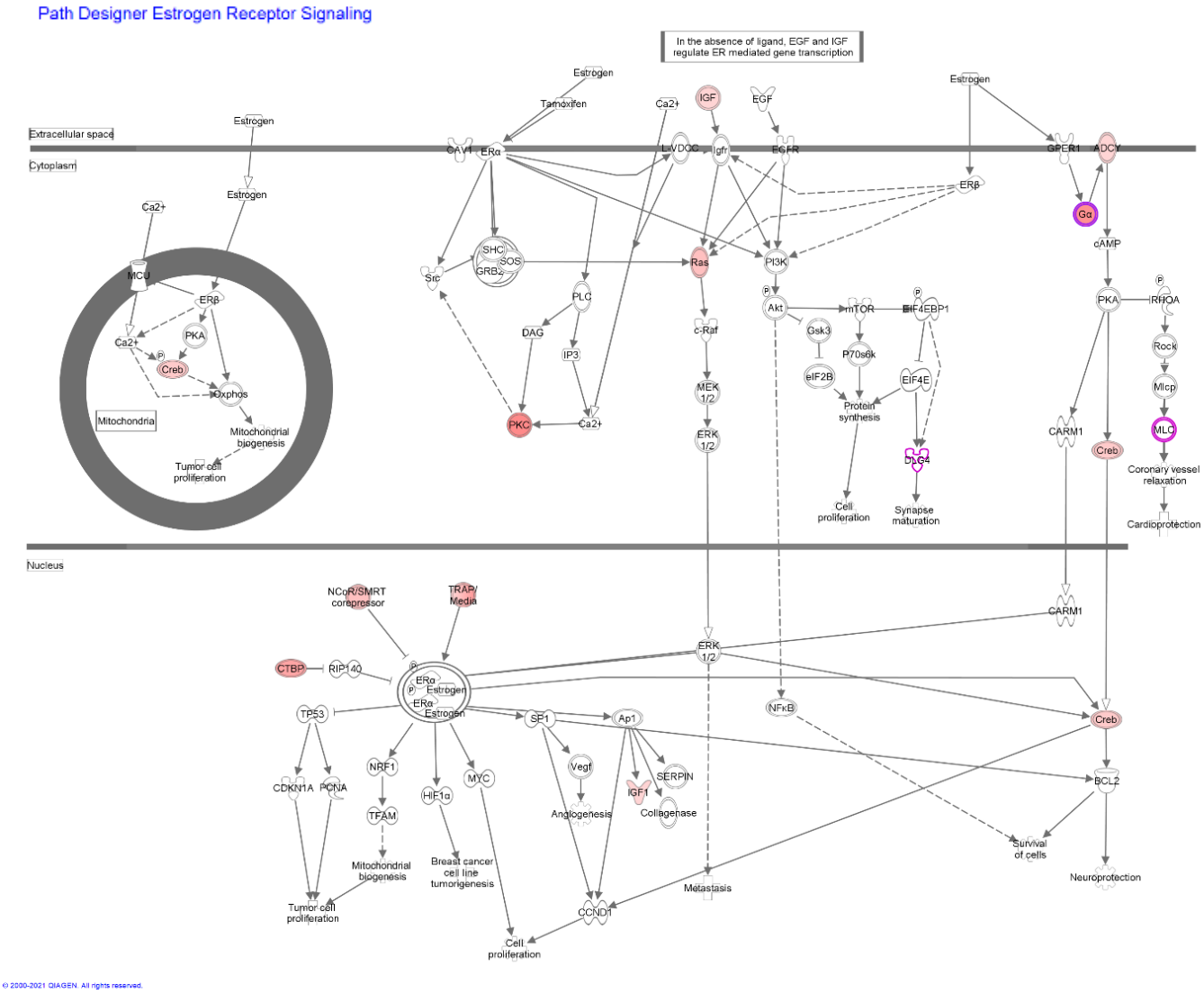

Figure 3 *Preferentially essential genes in CDKN2A and NF1 knockout cell lines in the estrogen receptor pathway* The genes found to be enriched in the *CDKN2A* and *NF1* knockout cell lines in the estrogen receptor signaling pathway in the CRISPR-Cas-9 dataset (red) and the RNAi dataset (purple) from IPA (QIAGEN Inc.). Enriched pathway molecules included G-alpha, IGF, IGF1, Ras, PKC, ADCY, CREB, CTBP, TRAO/Media, and NCoR/SMRT corepressor. RNAi-associated pathway molecules included G-alpha, DLG4, and MLC.

associated with the selectively essential genes by three standard deviations in the CRISPR and RNAi datasets compared to the average association in the datasets (Figure 2). Many proteins found in this signaling pathway are all known components in the NF1 condition, including Ras, a protein directly regulated by neurofibromin (Hannan et al., 2006). Previous work has shown that NF1 tumors often present PKCε, and ADCY1 (Supplementary Table 2). IGF-1 is known to be associated with NF1, and IGF-1R has been found to be upregulated in NF1-associated peripheral nerve sheath tumors (Friedrich et al., 2007). The IGF1R is already currently being studied as a potential therapeutic target for MPNSTs (Yang and Zhang, 2012). The progesterone receptor (PGR) is another target with a multitude of known drugs with an established association with NF1 (McLaughlin

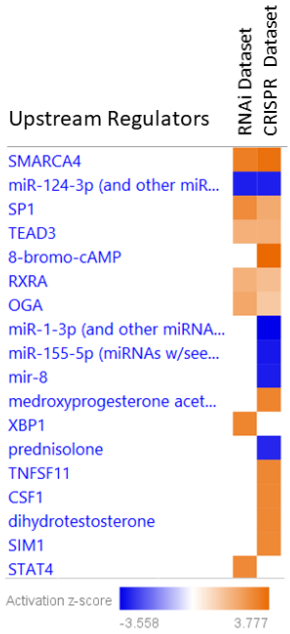

Figure 4 *Comparison of upstream regulators of dependent genes in NF1 and CDKN2A knockouts between RNAi and CRISPR datasets.* Ingenuity Pathway Analysis of upstream regulators of selectively essential genes in *NF1* and *CDKN2A* knockouts in CRISPR-Cas-9 dataset (left column) and RNAi datasets (right column). The activation Z-score corresponds to if the upstream regulator was over (orange) or under-represented (blue). SMARCA4 was significantly overexpressed with a Z-score of more than 3.5, while miR-124-3p was significantly under expressed with a Z-score of less than -3. SMARCA4 products are found significantly more frequently in the selectively essential genes from both datasets compared to average upstream regulator products, while miR-124-3p products are found significantly less.

Table 2).

The ADCY1 target that was identified encodes an adenylyl cyclase which converts ATP into second messenger cyclic AMP (cAMP) (Dessauer et al., 2017). This secondary messenger transduces many signals to activate metabolism, gene regulation, growth factors, and immune function (Patra et al., 2021). While the ADCY1 enzyme plays a significant role in many cell functions, it has also been specifically associated in the literature with *NF1*. In addition to acting as a suppressor of Ras as previously described, studies have found neurofibromin to regulate the cyclic AMP pathway through adenylyl cyclase (Tong et al., 2002). *NF1* associated MPNSTs were found to have twice the levels of basal cAMP as normal human Schwann cells (Dang and De Vries, 2011). This would be a complex pathway to target but shows significant potential as a tumor specific mechanism to inhibit.

The other two targets identified from the IPA analysis have a lesser-known association to NF1. Cyclin-Dependent Kinase 8 (CDK8) has been researched for its role in transcription, and inhibitors for this kinase have been investigated for anticancer potential (Solum et al., 2020). However, this enzyme has not been studied in the context of the NF1 condition except for its association with a certain micro RNA (miRNA) that functions as an RNA silencing regulator (MacFarlane and Murphy, 2010). Neurofibromin

Neurofibromin was found to be regulated by miR-107, and increased expression of this microRNA leads to increased cell proliferation through neurofibromin suppression (Wang et al., 2016). This microRNA also regulates CDK8 in other cancers, and miR-107 inhibitor lowered both CDK8 and cell proliferation in gastric cancers. These results suggest CDK8 might be a potential inhibitor of *CDKN2A* and *NF1* null cancer cells.

The PKCε target that was identified has not been studied in association with the NF1 genetic condition, but has been shown to turn on NFκB and tumorigenic signaling (Körner et al., 2013). This enzyme has been separately studied and targeted for its role on ethanol synaptic transmission as a potential treatment for drug alcohol use (Blasio et al., 2018). This presents another new potential drug for targeting this tumorigenic pathway in NF1 associated tumors.

Upstream regulators associated with selectively essential genes in NF1 and CDKN2A deficient cell lines

Along with the canonical pathway products identified in the analysis, several upstream regulators were found to be significantly associated with the selectively essential genes in the *NF1* and *CDKN2A* null cell lines (Figure 4). The *SMARCA4* gene encoding chromatin-remodeling SWI/SNF complex was highly enriched in both the CRISPR and RNAi datasets. This gene is often overexpressed in tumors, where high expression is are associated with a poor prognosis (Guerrero-Martínez and Reyes, 2018). Interestingly, the loss of the *SMARCA4* gene along with CDK4/6 has been found to be lethal in lung cancer cells (Xue et al., 2019). This result suggests that SMARCA4 might be a good target to test synthetic lethality in combination with the loss of NF1 and CDKN2A. The other most strongly correlated upstream pathway was the under-representation of miR-124-3p in the datasets. This tumor-suppressing microRNA suppresses growth in a variety of cancers, and has recently been investigated as a potential target for aggressive glioblastoma (Liu et al., 2018; Zhang et al., 2018). Activating this tumor-suppressing pathway to influence the selectively essential genes in MPNST precursors would be another strategy to investigate new treatments of this condition.

Limitations

While these targets seem to be promising for targeting *NF1* and *CDKN2A* null cancer cell lines, the origin of the gene dependency cell lines should be considered as a potential confounding factor. Most of the cell lines of interest were taken from skin melanoma cancers (Supplementary Table 1). The associations that have been found in this analysis could be picking up associations between skin cancers instead. A future analysis of only skin melanoma cancers would be an important next step to confirm whether these associations found were due to the *NF1* and *CDKN2A* null mutations, or as a result of the lineage of the cancer cell lines studied.

Conclusion

Targeting and preventing NF1-associated neurofibromas from transforming into MPNSTs is an important strategy to reduce morbidity and mortality for NF1 patients. The loss of the tumor suppressor genes NF1 and CDKN2A has been associated with tumor progression into atypical neurofibromas that have a high potential of malignant transformation. As cells loose certain

genes, they can become reliant on other cellular pathways, which can be identified through molecular screening and be targeted for treatment. Here, the computational screening of cancer cell line libraries for pathways overrepresented in *NF1* and *CDKN2A* deficient cancer cells uncovered several targets. Estrogen receptor signaling was the most significantly correlated canonical pathway, with four current drugs targeting the selectively essential genes of the *NF1* and *CDKN2A* knockouts. The identification and research of novel targets for MPNST precursors can be followed by in vitro and in vivo experiments screening the identified compounds against NF1 null Schwann cells. Future experiments on this topic for empirical data on the potential use of these drugs will hopefully help prevent future malignancies for patients with NF1.

Methods

Identification of NF1 and CDKN2A Null cell lines

The Cancer Dependency Map Project data was used for filtering out cell lines containing deleterious mutations for *Nf1* and *CDKN2A*. RNAi and CRISPR data sets of cancer gene dependency studies were imported into R studio version 1.3.1073 (Dempster et al., 2019; Meyers et al., 2017; RStudio Team, 2020). The R studio data package "DepMap" from the Bioconductor Project Package Manager was used to upload the data from DepMap (Laurent Gatto and Theo Killian, 2020). A data frame was created to only include cell lines with deleterious mutations in *Nf1* and *CDKN2A* from the mutation calls "EH3457" dataset (cell lines of interest) (Supplementary Table 1).

Finding genes essential in only NF1 and CDKN2A Knockout cancer cell lines

The cell lines of interest were analyzed for gene dependencies from a CRISPR and RNAi dataset in R studio using the datasets "EH2261" and "EH2260". The CRISPR dataset was filtered to only contain the cell lines of interest. To compare the gene dependencies in these cell lines of interest to a general gene dependency average, a data frame of average gene dependencies of each gene in the full CRISPR dataset was calculated. The data frames were then joined according to gene name, and the difference between the gene dependencies of the cell lines of interest and the general averages were calculated. The list of genes from the cell lines of interest were then sorted according to the difference in gene dependencies. The list was then filtered of any genes that in the full dataset would be considered essential across all the cell lines, by eliminating genes with an average gene dependency less than -0.5 (Joshua Dempster, 2020). The top 500 genes with the largest differences in gene dependencies were then exported for both the CRISPR and RNAi datasets (Supplementary Table 3 and 4).

Ingenuity Pathway Analysis

Analysis of pathways was done using Ingenuity Pathway Analysis (IPA) (Krämer et al., 2014; QIAGEN Inc.). Both lists of genes were uploaded into IPA with the absolute differences between the gene dependency used as a measure of expression. The output analysis of the CRISPR-Cas-9 dataset and the RNAi dataset were then investigated using a comparative analysis.

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Supplementary Data

DepMap ID	Lineage	Subtype	Gene	Variant Class	Variant Type	Deleterious?	Annotation	Genome Change	cDNA Change	Codon Change	Protein Change
ACH-000971	Colorectal	Adenocarcinoma	NF1	Frame_Shift_Del	DEL	TRUE	damaging	g.chr17:29553478delC	c.2027delC	c.(2026-2028)accfs	p.T676fs
ACH-001554	Eye	Uveal Melanoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29557942A>G	c.3196A>G	c.(3196-3198)Aga>Gga	p.R1066G
ACH-000219	Skin	Melanoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29562790G>A	c.3870G>A	c.(3868-3870)aaG>aaA	p.K1290K
ACH-000934	Breast	Carcinoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29562790G>A	c.3870G>A	c.(3868-3870)aaG>aaA	p.K1290K
ACH-002001	Skin	Melanoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29562790G>A	c.3870G>A	c.(3868-3870)aaG>aaA	p.K1290K
ACH-002002	Skin	Melanoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29562790G>A	c.3870G>A	c.(3868-3870)aaG>aaA	p.K1290K
ACH-002003	Skin	Melanoma	NF1	Splice_Site	SNP	TRUE	damaging	g.chr17:29562790G>A	c.3870G>A	c.(3868-3870)aaG>aaA	p.K1290K
ACH-000987	Skin	Melanoma	NF1	Nonsense_Mutation	SNP	TRUE	damaging	g.chr17:29576033C>T	c.4006C>T	c.(4006-4008)Cag>Tag	p.Q1336*
ACH-000987	Skin	Melanoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971120G>A	c.85C>T	c.(85-87)Cga>Tga	p.R29*
ACH-000219	Skin	Melanoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971153C>A	c.52G>T	c.(52-54)Gag>Tag	p.E18*
ACH-000934	Breast	Carcinoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971153C>A	c.52G>T	c.(52-54)Gag>Tag	p.E18*
ACH-002001	Skin	Melanoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971153C>A	c.52G>T	c.(52-54)Gag>Tag	p.E18*
ACH-002002	Skin	Melanoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971153C>A	c.52G>T	c.(52-54)Gag>Tag	p.E18*
ACH-002003	Skin	Melanoma	CDKN2A	Nonsense_Mutation	SNP	TRUE	damaging	g.chr9:21971153C>A	c.52G>T	c.(52-54)Gag>Tag	p.E18*
ACH-001554	Eye	Uveal Melanoma	CDKN2A	Frame_Shift_Del	DEL	TRUE	damaging	g.chr9:21971157_21971164delGCCGTGGA	c.41_48delTCCACGGC	c.(40-48)ctccacggcfs	p.LHG14fs
ACH-000971	Colorectal	Adenocarcinoma	CDKN2A	Frame_Shift_Ins	INS	TRUE	damaging	g.chr9:21974758_21974759insC	c.68_69insG	c.(67-69)ggtfs	p.G23fs

Supplementary Table 1

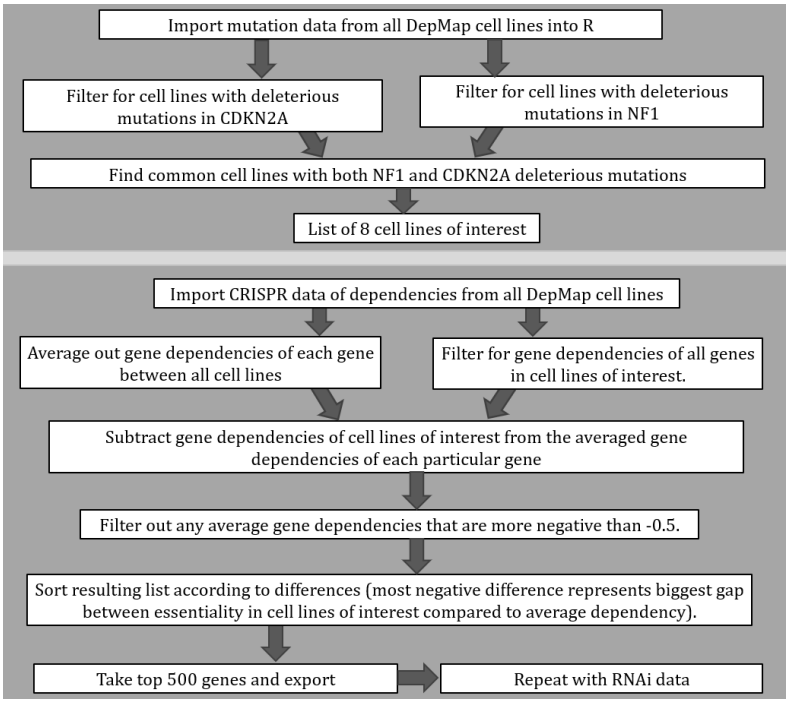
List of cell lines from DepMap with deleterious mutations in both *NF1* and *CDKN2A*, filtered using R.

Symbol	Entrez Gene Name	Entrez Gene/Gene Symbol - human (HUGO / HGNC / Entrez Gene)	Absolute difference of Individual gene dependency and average dependency	Individual gene dependency in cell line	Average gene dependency among all cell lines	Location	Type(s)	Drug(s)
ADCY1	adenylate cyclase 1	ADCY1	0.134	-0.09145	0.042598	Plasma Membrane	enzyme	
CCNC	cyclin C	CCNC	0.227	-0.52387	-0.29647	Nucleus	other	
CDK8	cyclin dependent kinase 8	CDK8	0.24	-0.35795	-0.11804	Nucleus	kinase	alvocidib,SEL120,senexin B
CREB1	cAMP responsive element binding protein 1	CREB1	0.168	-0.27684	-0.10859	Nucleus	transcription regulator	
CTBP2	C-terminal binding protein 2	CTBP2	0.289	-0.63576	-0.34714	Nucleus	transcription regulator	
GNAQ	G protein subunit alpha q	GNAQ	0.352	-0.60203	-0.25047	Plasma Membrane	enzyme	
IGF1	insulin like growth factor 1	IGF1	0.136	-0.20481	-0.069	Extracellular Space	growth factor	BI 836845,MEDI-573
MED13	mediator complex subunit 13	MED13	0.178	-0.16478	0.012861	Nucleus	transcription regulator	
MED16	mediator complex subunit 16	MED16	0.235	-0.38385	-0.14845	Nucleus	transcription regulator	
MED23	mediator complex subunit 23	MED23	0.325	-0.51843	-0.1938	Nucleus	transcription regulator	
MED24	mediator complex subunit 24	MED24	0.193	-0.5402	-0.34694	Nucleus	transcription regulator	
NCOR1	nuclear receptor corepressor 1	NCOR1	0.2	-0.2992	-0.09933	Nucleus	transcription regulator	
PGR	progesterone receptor	PGR	0.132	-0.08778	0.044616	Nucleus	ligand-dependent nuclear receptor	17alpha-hydroxyprogesterone caproate,asoprisnil,beta-estradiol/progesterone,danazol
PRKCE	protein kinase C epsilon	PRKCE	0.374	-0.54132	-0.16774	Cytoplasm	kinase	ingenol mebutate,protein kinase C-epsilon activator
RALA	RAS like proto-oncogene A	RALA	0.199	-0.30679	-0.10761	Cytoplasm	enzyme	
THRAP3	thyroid hormone receptor associated protein 3	THRAP3	0.148	-0.20457	-0.05689	Nucleus	transcription regulator	

*PGR drug targets continued: desogestrel,desogestrel/ethinyl estradiol,dienogest,drospirenone,drospirenone/ethinyl estradiol,dydrogesterone,estradiol cypionate/medroxyprogesterone acetate,estradiol/levonorgestrel,estradiol/norethindrone acetate,ethinyl estradiol/ethynodiol diacetate,ethinyl estradiol/etonogestrel,ethinyl estradiol/levonorgestrel,ethinyl estradiol/norelgestromin,ethinyl estradiol/norethindrone,ethinyl estradiol/norgestimate,ethinyl estradiol/norgestrel,ethynodiol diacetate,etonogestrel,fluorine F 18 fluoro furanyl norprogesterone,leuprolide acetate/norethindrone acetate,levonorgestrel,lonaprisan,medroxyprogesterone acetate,medroxyprogesterone acetate/megestrol acetate,megestrol acetate,megestrol acetate/tamoxifen,mifepristone,misoprostol,mometasone furoate,nestorone,norelgestromin,norethindrone,norethindrone acetate,norgestimate,norgestrel,onapristone,progesterone,progesterone receptor antagonist,tanaproget,telapristone acetate,tosagestin,ulipristal,ulipristal acetate

Supplementary Table 2

Estrogen Receptor Signaling product and drug targets from the estrogen receptor signaling pathway, obtained from QIAGEN Ingenuity Pathway Analysis.



Supplementary Figure 1

R studio workflow for filtering NF1 and CDKN2A Knockouts, and finding genes essential to only those cancer cell lines

Supplementary Table 3 (attached)

Top 500 genes from the CRISPR-Cas-9 dataset with the greatest differences between gene dependencies in the NF1 and CDKN2A knockout cell lines compared to all cell lines. Genes that were considered essential overall (gene dependency less than -0.5) were eliminated.

Supplementary Table 4 (attached)

Top 500 genes from the RNAi dataset with the greatest differences between gene dependencies in the NF1 and CDKN2A knockout cell lines compared to all cell lines. Genes that were considered essential overall (gene dependency less than -0.5) were eliminated.