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RNAi as a tool for target discovery in early pharmaceutical research

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The pharmaceutical industry is currently faced with increasing pressure due to patent expirations for block busters, healthcare reforms with strained budgets and growing demands for approval by administrative organizations like the FDA and the EMA. High attrition rates especially in the later expensive stages of the drug development process ask for thoroughly validated drug targets at the beginning of such projects. The great potential of RNA interference strategies toward reaching this goal is outlined in this article.

1. Introduction

The pharmaceutical industry is currently faced with increasing pressure due to patent expirations for block busters, healthcare reforms with strained budgets and growing demands for regulatory approval by administrative organizations like the FDA. Costs for drug development are steadily increasing while the number of novel drugs entering the market has decreased over the past 20 years. This is in contrast to the number of novel compounds in development, which increased significantly over the last decade. The high attrition rate especially in the later expensive stages of the drug development process poses a significant challenge for pharmaceutical development and emphasizes the need for thoroughly validated drug targets as starting points of drug development.

More than 15 years ago, scientists discovered RNA interference (RNAi) as a powerful tool to knockdown gene expression in a highly targeted way. Since, it has become an invaluable so-called reverse genetics tool to decipher gene functions and discover novel targets in a phenotype-specific manner. The publication of the human reference genome in 2001 formed the basis for high-throughput RNAi screens since it was now possible to target any known transcribed gene in a sequence-specific manner. That enabled researchers not only to functionally annotate genes but also to improve knowledge for understanding the functional relevance of disease-associated genetic alterations. RNAi is now a standard tool for target identification and validation in early drug discovery. Here, we highlight the importance of functional screening and validation in pharmaceutical target discovery and give an overview of target discovery and validation strategies utilizing RNAi techniques.

2. RNA interference as a tool for target discovery and validation

A critical step in the drug development pipeline process, prior to setting up and executing ultra-high throughput compound screens is the identification of putative targets (Fig. 1). More so, it has to be shown that the targets indeed play an essential causative role in a disease and target inhibition is able to suppress pathophysiologically relevant disease mechanisms. Furthermore, novel approaches are aiming at personalized treatment strategies like customized diagnosis and treatment tailored to the needs of the individual patient. Therefore, molecular markers predicting the response of an individual patient to the drug are also an important aspect of target research. The growing knowledge of the genetic and molecular basis of diseases is a prerequisite for this approach.

In the initial phase of drug development, it is essential to identify the right model systems of disease and to show that addressing a given target will be efficacious in this specific model. The ability to decipher the human genome has had a tremendous impact on the understanding of a number of diseases, for finding novel biomarkers and targets, as well as for the identification of drug resistance mechanisms. However, for the verification of novel targets it is necessary to associate disruption of the target gene with functional (loss-of-function) phenotype in order to infer possible treatment options. To do so, the discovery of RNAi and the harnessing of this method as a research tool was one of the major achievements of the last decade.

RNAi is a term for various gene regulatory mechanisms. It was observed for the first time in 1990 in transgene petunia when overexpression of the chalcone synthase gene resulted in an unexpected repression of anthocyanin biosynthesis (Napoli et al. 1990). The effect was termed co-suppression and caused a phenotype of white flowers rather than the expected increase of intensity of the purple blossom color. The mechanism of this unexpected outcome remained unclear until 1998 when Fire and Mello were able to show that the introduction of double-stranded RNA (dsRNA) into the nematode *Caenorhabditis elegans (C. elegans)* was critical for the suppression of gene expression (Fire et al. 1998) (Fig. 2). This observation was honored with the Nobel Prize in Physiology or Medicine in 2006. The simplicity of applying RNAi to worms by solely feeding dsRNA, combined with the availability of its genome sequence, made *C. elegans* an exceptionally well-suited model organism enabling chromosome-wide (Fraser et al. 2000) and later genome-wide screens (Vastenhouw et al. 2003). The rapid impact of the results in *C. elegans* led to the adaption of the screening method to other model organisms such as *Drosophila melanogaster* in a cell-based manner (Boutros et al. 2004). In 2001, Elbashir and Tuschl were able to induce RNAi in mammalian cells by the introduction of short (21 nt) dsRNAs (Elbashir et al. 2001). The application of this principle led to a wealth of information on so

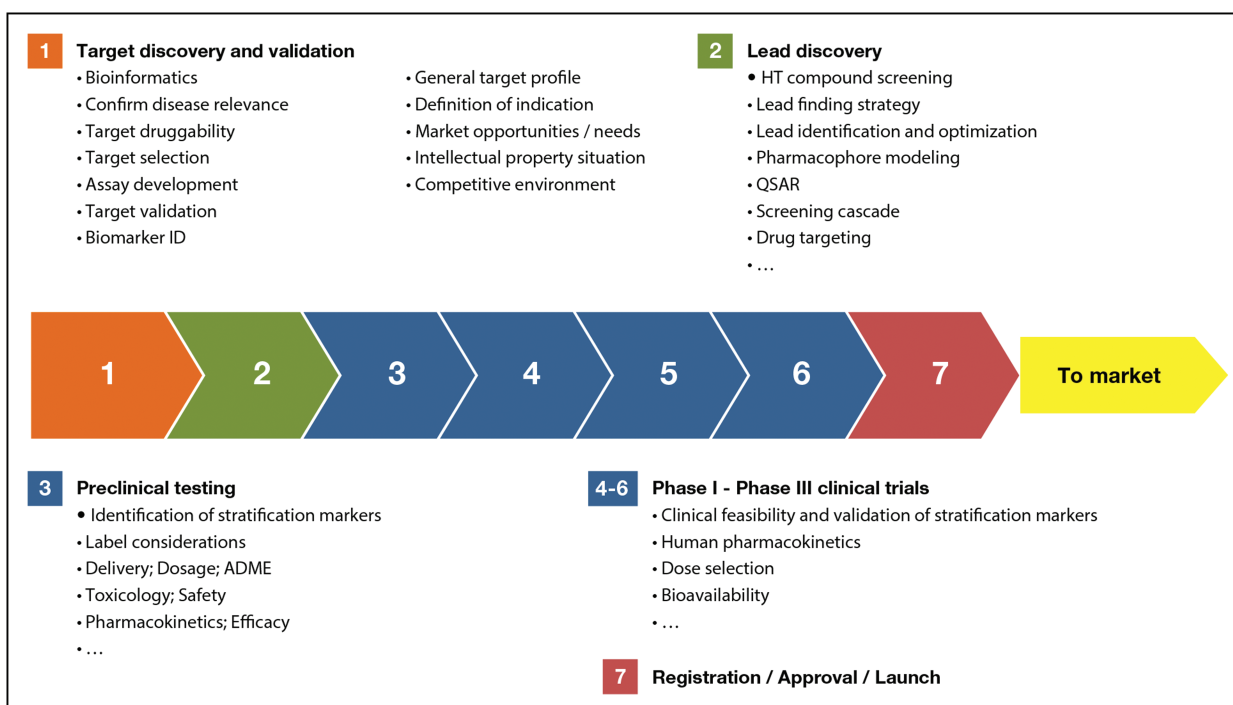

Fig. 1: Drug development pipeline. Target discovery and validation represent the initial step of the drug development pipeline. An important task in this first step is the identification and validation of the role a target plays in a disease- specific context. All later steps depend on the appropriateness of the chosen target and emphasize the importance of a thorough target validation. Biomarkers: indicators (e.g. genetic alterations, activities of enzymes, concentration of compounds in the blood or expression status of genes) that can be measured to identify pathogenic conditions or a cellular response after a treatment. QSAR: Quantitative structure-activity relationship – a set of variables, describing molecular properties of chemical or biological structures used to predict activities by regression or classification models. ADME: absorption, distribution, metabolism and excretion – description of processes leading to disposition of a drug in an organism after administration. Stratification marker: biomarkers that are used to predict likelihood of a beneficial or rather unfavorable response after administration of a therapeutic compound. HT: high-throughput.

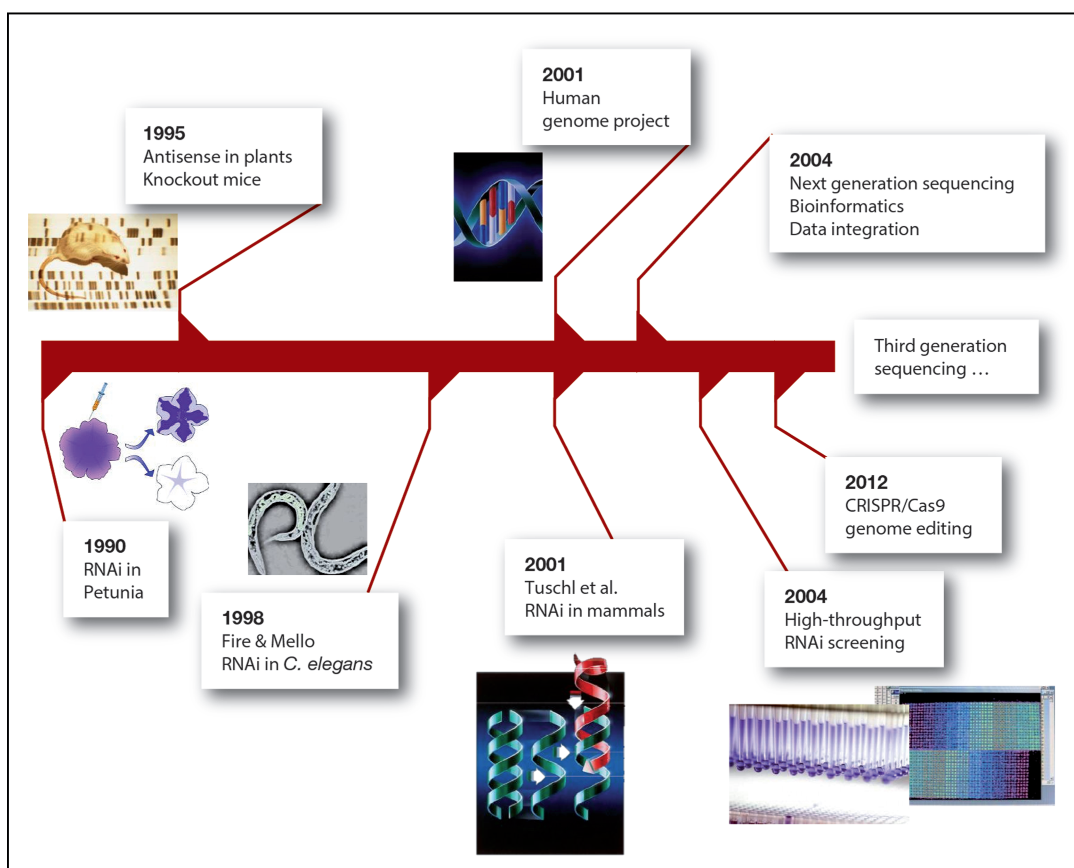

Fig. 2: Milestone discoveries in functional genomics research. In 1990, transgene Petunia blossoms were found to respond differently than expected to the insertion of a gene that should increase color intensity. Instead, a decrease in color intensity was observed. This was the first observation of RNAi. Eight years later, Fire and Mello deciphered the mechanism behind RNAi in the worm Caenorhabditis elegans and in 2001 Tuschl et al. were able to induce RNAi in mammalian cells with short double-stranded siRNAs. Together with other important discoveries these technologies enable us to decipher gene functions in a disease context.

far unknown gene functions and further revealed genes concertedly acting in cellular pathways. RNAi technology accelerated the gain of knowledge in many areas, particularly in oncologic research. Not surprising, the gained information raised reasonable expectations for the application of RNAi to the treatment of diseases caused by gene malfunction (Micklem and Lorens 2007).
There are two major applications of RNAi that have been used so far in pharmaceutical research: target identification and target validation. For target identification, knowledge of the complete human genome allows screening for phenotypes of interest in a large-scale fashion by inhibiting gene functions in a group of specific genes (e.g. all known kinases) or all known genes in parallel. Especially in oncologic research this approach is of high importance (Quon and Kassner 2009). Utilizing reverse genomics approaches in established cancer cell line models allows for genome-wide screening for the detection of vulnerabilities in cell models in a relatively rapid and cost-saving manner. For target validation, RNAi is used to identify and characterize appropriate cell models as well as to confirm the validity of the targeting approach in a wider panel of cell models that represent the genetic variability of tumor subtypes found in patient populations.

3. siRNA- and shRNA-based screening for target identification

Loss-of-function experiments by knocking out genes have been envisioned in the early 1980 s and were a source for the identification of gene functions as laboratory standard methods for the last two decades. Earlier approaches utilized insertional mutagenesis or homologous recombination to induce loss-of-function phenotypes in different model organisms (Kile and Hilton 2005). While those experiments gave highly valuable insights into many hitherto unknown gene functions and helped understanding disease relevance of dys-regulated genes, they are too laborious to be performed in a high-throughput fashion. With the knowledge of the sequence of each protein-coding gene and the discovery and subsequent utilization of RNAi, scientists were equipped with a tool to disrupt gene functions in screens in high parallelization (Vanhecke and Janitz 2005).
The forerunner of *in vitro* loss-of-function phenotyping was the antisense-technology discovered in 1978 (Zamecnik and Stephenson 1978), which is described in more detail in the article by Grünweller & Hartmann in the current issue.
Generally, RNAi leads to the specific knockdown, i.e. the repression, of a targeted mRNA. Different strategies for inducing such a repression exist. Here, we highlight the most common approaches of inducing RNAi in cells, i.e. by small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs). Both strategies differ significantly in terms of required equipment, available assays and fields of application.
High-throughput siRNA experiments are performed in an arrayed format using 96, 384 (standard) or 1536 well plates. A higher miniaturization increases the requirement for precision and automation and also restricts possibilities in terms of phenotypic assays / readouts. The standard transfection method for siRNA is lipofection (liposome transfection). Advances in the understanding of RNAi biology as well as bioinformatic analyses have shown that screening a single target by several different siRNAs in parallel allows minimization of false positive and false negative results significantly (Echeverri et al. 2006). Thus, every gene should be targeted by different siRNAs that, in the best case, show all comparable phenotypes. Thereby two different strategies can be applied: every single siRNA is transfected on its own or multiple siRNAs against one target are pooled (transfected in one single well). The aforementioned bioinformatics analyses have shown that it is beneficial to screen with single siRNAs, as this facilitates evaluation of individual siRNA performance and thus helps in identifying false positives easier. In contrast, using pooled siRNAs (i.e. three or four siRNAs which target the same gene in one well) complicate analysis as potential off-target effects accumulate and those effects cannot be assigned to single siRNAs but instead must be deciphered in further experiments. Performing a genome-wide screen in triplicates for two conditions (e.g. a compound vs. negative vehicle control or isogenic cell lines, i.e. engineered cell lines that are genetically identical except for a specific alteration, e.g. a mutation) would therefore require ($\sim$ 21.000 genes x 3 siRNAs x 3 replicates) x 2 / 384 = 940 plates of 384 wells. SiRNA screens thus have high requirements for automation (by robot liquid handling and automated microscopy) (Conrad and Gerlich 2010), laboratory infrastructure, data tracking (by barcoded and automatically tracked well plates) and subsequent data analysis (data storage and analysis) (Boutros et al. 2006). Several commercial libraries of pooled or single siRNAs are available that target the entire human genome ($\sim$ 21.000 genes), the druggable genome ($\sim$ 8.000 – 10.000 genes) or specific subsets of genes (e.g. $\sim$ 700-800 kinases).
For validation purposes of previously identified hits, commercially available siRNAs or custom-synthesized siRNAs can be utilized. In an industrial setting, up to 20 different siRNAs, preferably from different vendors, are used for validation of a single target. This significantly increases confidence in the validity of target-dependent phenotypic effects, if the majority of siRNAs induce the same phenotype. In addition to replicating the phenotype, the knockdown is confirmed by qRT-PCR on the mRNA level and by Western Blotting on the protein level.
The advantage of siRNAs is the ease of transfection and the availability of a large number of specific assays (see below) and optimized cell lines. A drawback of siRNAs compared to shRNAs is the expense in terms of needed infrastructure and the relatively high costs (e.g. for transfection reagents and siRNA libraries). Furthermore, siRNAs allow only transient transfection and are thus not useful for long-term experiments. In general, the readout in siRNA experiments is recorded 48 to 72 h after transfection. Depending on the doubling time of the specific cell line in use, siRNAs become diluted and phenotypic effects decrease. In contrast, shRNAs allow for a much higher parallelization by pooling libraries (Hu and Luo 2012). Due to the genomic integration and constitutive or induced expression of shRNAs, long-term experiments up to a few weeks can be conducted. The delivery of shRNAs is carried out by lentiviral vectors. Such vectors contain a barcode that is specific to every shRNA, selection markers, a constitutive or inducible promoter and a sequence encoding the shRNA. After virus infection, the plasmid is integrated into the genome of the respective cell line and is either expressing the shRNA constitutively or after induction of the promoter. The expressed shRNA hairpin structure mimics a pri-miRNA substrate that is processed to an shRNA by the protein Drosha (Fig. 3). The shRNA is exported to the cytosol and finally processed to an siRNA. A large number of cell lines can be infected by a pool containing genome-wide shRNA sequences in parallel. This can be done in petri dishes or cell culture flasks and is thus less demanding to laboratory infrastructure when compared with siRNAs.
After infection, the cells are cultivated for a specific duration depending on the doubling time. The readout for shRNA screens is limited and in general proliferation screens are conducted. By combining shRNA screens with compound treatment, the use of isogenic cell lines, or double knockdowns (e.g. siRNA treatment in shRNA expressing cell lines), complex biological questions can be answered (e.g. synthetic lethal dependencies,

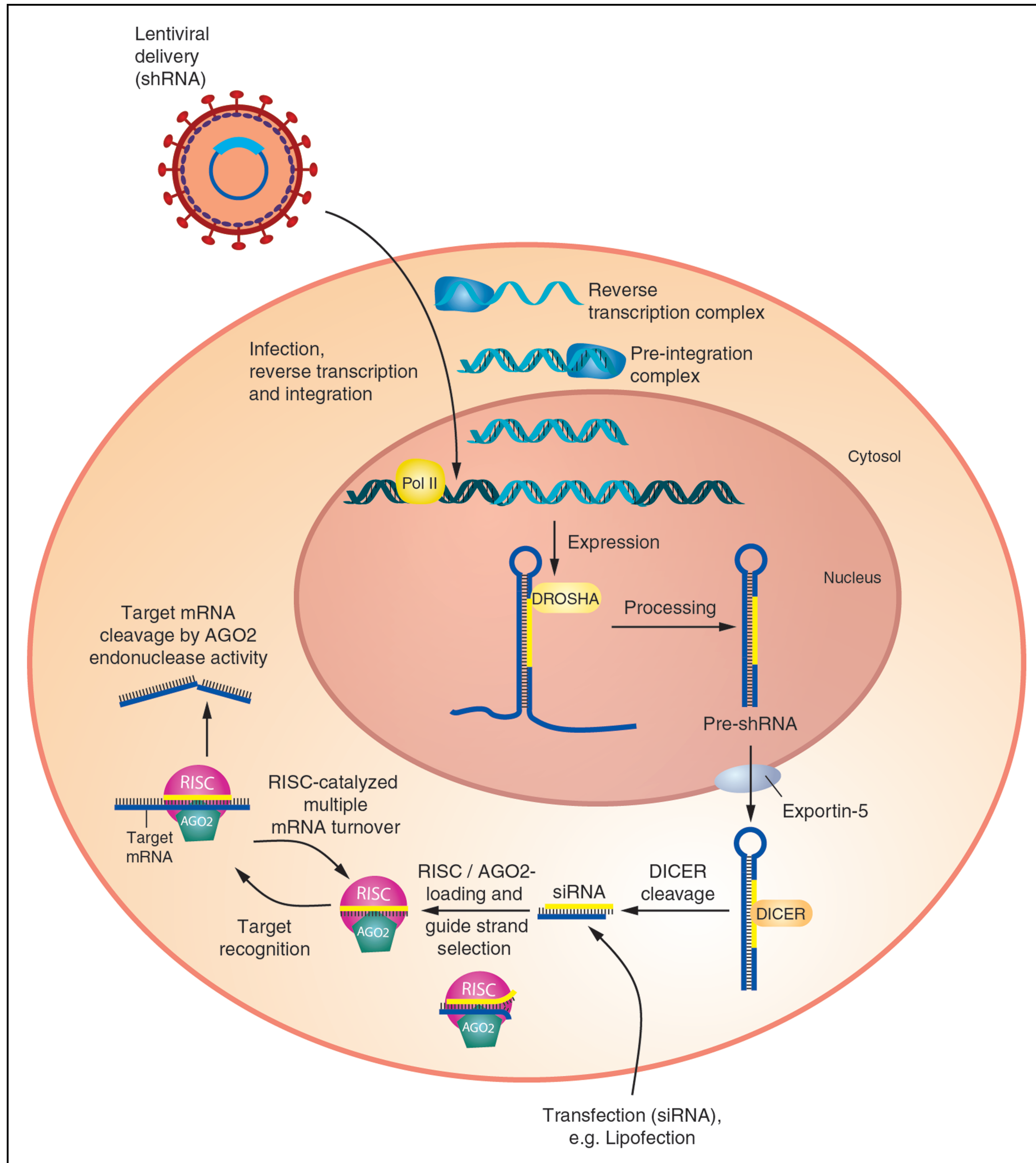

Fig. 3: The RNAi pathway offers different points of interacting with this endogenous regulatory mechanism. The two most common forms of inducing RNAi in cells are the lentiviral delivery of shRNA expression plasmids or the transfection (most often by lipofection, i.e. the delivery of siRNA in lipid vesicles) of naked dsRNAs with 2-nt overhangs at the 3' ends.

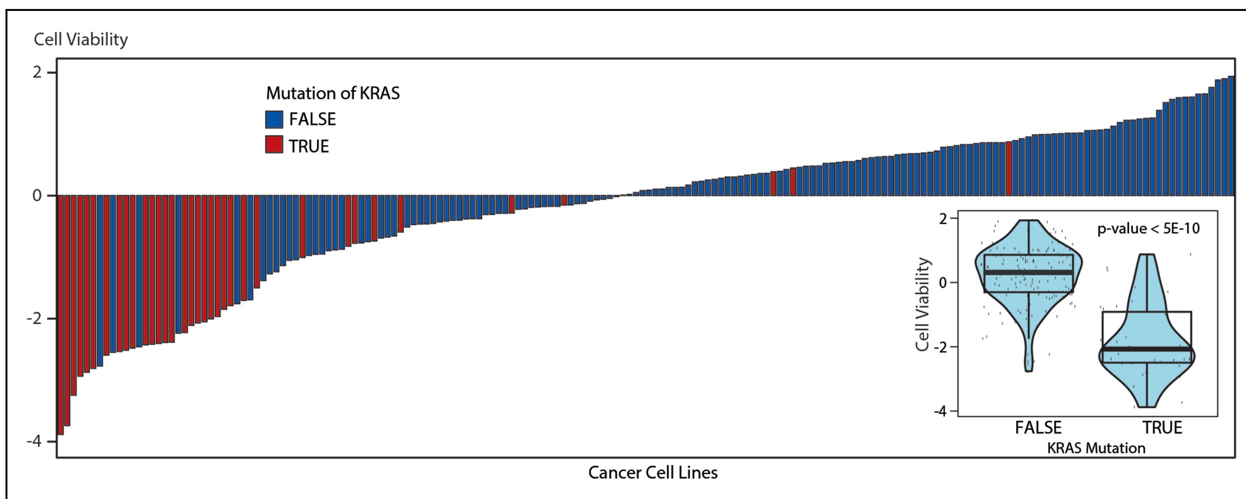

Fig. 4: Example for the correlation of functional cell viability data with oncogenomics data. A KRAS mutation sensitizes a cell line towards the knockdown of KRAS, a phenomenon that is well studied and known as an oncogenic addiction. The viability data is derived from massive parallel shRNA screening in several hundred cancer cell lines. The mutation data is provided by the CCLE (Cancer Cell Line Encyclopedia) [26].

see below). The basic principle for determining inhibitory or stimulatory effects on proliferation after knockdown of a gene by shRNAs is based on the depletion or enrichment, respectively, of the barcode-carrying plasmid from the cell population. After termination of the experiment, total DNA is extracted from the cell culture and the amount of individual plasmid-encoded shRNAs is determined by either microarray technology or by next generation sequencing (NGS). Thereby, a short sequence of a genomically integrated shRNA construct is complementary to a primer used in the sequencing library. This sequence is followed by a unique barcode sequence that identifies an integrated shRNA unambiguously and thus enables a quantification of the presence of the shRNA in the cell pool after termination of the experiment. The reason for NGS as the method of choice is that microarrays lack the sensitivity necessary for detecting marginal differences when only few molecules of a barcode are remaining in the cell population (Sims et al. 2011). The remaining barcodes are normalized to the initial pool. Barcodes that are not detectable after termination of the experiment are classified to have targeted essential cell components, while unaltered relative amounts of a barcode indicates that knockdown of that gene has no phenotypic effect. This latter approach has been used to carry out one of the most comprehensive studies done so far to identify cancer vulnerabilities under the umbrella of the Project Achilles by the Broad Institute of Harvard and MIT in Boston (http://www.broadinstitute.org/achilles) (Cowley et al. 2014). They screened over 300 cell lines so far using a genome-wide shRNA library monitoring the effects of the shRNAs on viability of the cells. This allows not only the identification of essential genes per cell line but also the detection of subgroups of cells that share genetic alterations that render them sensitive towards the knockdown of specific targets. Cells are not sensitized towards the knockdown of those targets in the absence of the alteration (Fig. 4). Such so-called synthetic lethal correlations are a very important topic in pharmaceutical research as they guide the path to personalized therapeutic approaches (Azorsa et al. 2009; Luo et al. 2009). Synthetic lethality exploits interactions of two genes. These genes are said to be synthetic lethal if an alteration, e.g. an inactivating mutation, in either gene alone has no effect on cell viability but inactivation of both genes results in cell death. Isogenic cell lines are a perfect model to screen for synthetic lethal interactions by RNAi. Here, the goal would be to selectively kill the cell line that harbors an undruggable activated oncogene, but not the isogenic cell line lacking this alteration that leads to oncogene activation. A comprehensive review of the topic can be found in Paul et al. (2014).

When screening for novel targets, it is important to define the specific phenotype to screen for. Any cancer therapy aims to remove tumors or limit their growth. Thus, standard assays used in target validation aim at probing the consequences that a specific target gene knockdown has on the proliferation rate and on the induction of apoptosis. A plethora of assays is available to assess the health status of cells in culture, including fluorescence-based assays that measure intracellular redox potential or ATP levels as surrogates for the proliferation capacity of a cell. Some assays should be used with caution depending on the biological role of the target under investigation. Knockdown of targets in the field of tumor metabolism could easily affect ATP levels, giving rise to falsified results. One of the best methods for examining cellular proliferation is a direct count of the cell number before and after treatment using high-content analysis (Fuchs et al. 2010). Since standard 2D cell culture systems (i.e. cells grown as monolayers in petri dishes) lack many of the characteristics that cells are exposed to in the *in vivo* situation, more sophisticated experimental setups have been used in the past, including several 3D model systems where cells either

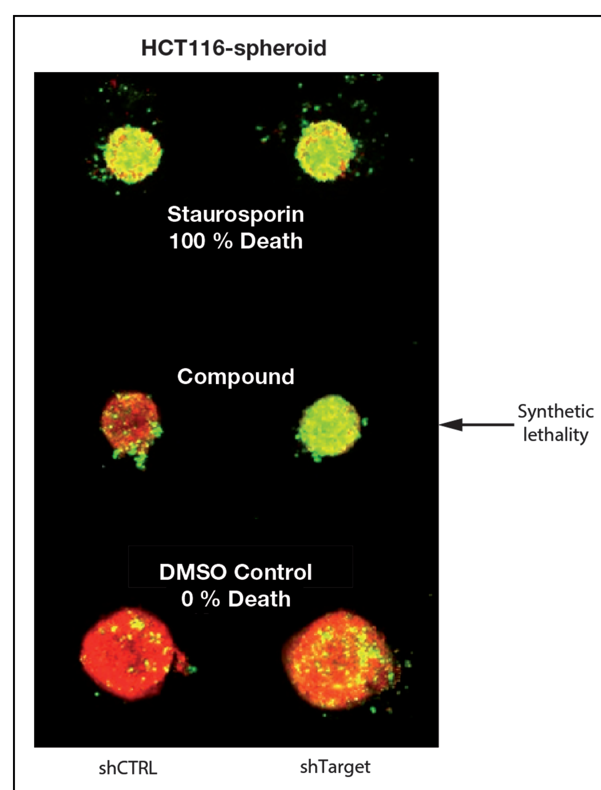

Fig. 5: 3D tumor spheroids represent a cell-based model that mimics the situation in a real tumor more appropriately. Green color indicates dead cells (Sytox Green assay counterstained by Hoechst). Spheroids targeted by shRNAs only (cells at the bottom) do not show increased cell death (negative control) while the combination of a specific compound and the targeting shRNA cause death of the cells inside the spheroid (synthetic lethality). Spheroids treated with very high concentrations of staurosporin (multikinase inhibitor that blocks ATP binding) do not survive independent of additional shRNA treatment (positive control, cells at the top). shCTRL is a non-targeting (so-called scrambled) control. (Figure generously provided by Patrick Steigemann and Gerrit Erdmann, Bayer Pharma AG).

grow as spheroids in a matrix or on a scaffold. 2D models, for example, do not recapitulate the gradients in nutrients and oxygen that are found in any tumor. 3D models are currently not accessible for siRNA experiments as transfection is only feasible for monolayer cell models in *in vitro* experiments. 3D models are used in combination with inducible shRNA systems. Wenzel et al. (2014) have developed a 3D high-content screening setup that identified compounds selectively killing dormant cancer cells in the inner core of spheroids. Here, the aim was to identify genes whose inhibition caused cell death in already formed spheroids rather than identifying genes that prevent formation of spheroids. This approach was done by screening compound libraries but it is also possible to use shRNAs in this setting. In this case, the assay is best performed using inducible rather than constitutive shRNA expression vectors so that the knockdown of a target can be induced once spheroids have formed (Fig. 5). Several other 3D models are being used to assess the importance of cancer targets. Amongst these are the soft-agar assays where cells seeded into semi-fluid agar are monitored until they have formed colonies, and the hanging drop model where cells grow in a colony at the bottom of a fluid drop (Foty 2011).

4. Validation strategies for target discovery

Personalized medicine in oncology aims at identifying and targeting specific cancer vulnerabilities of individual patients. To this end, there is an increasing trend in categorizing and treating cancer types according to their oncogenomic makeup (i.e.

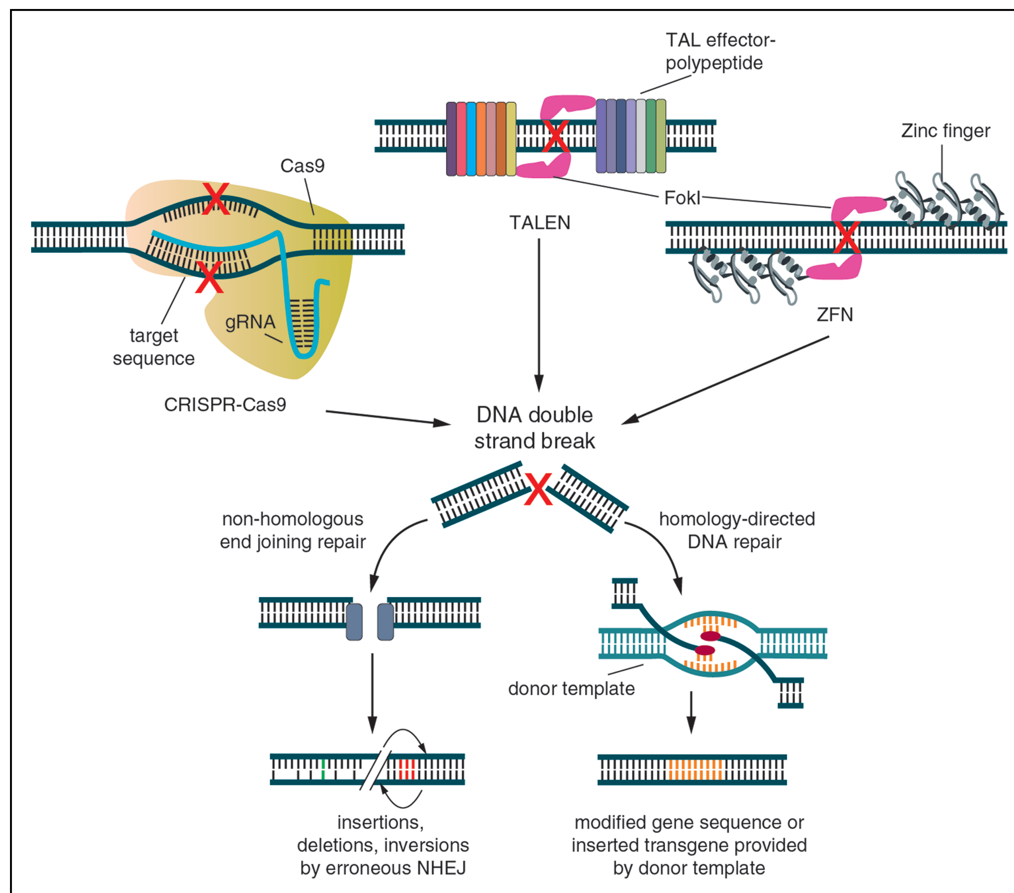

Fig. 6: Genome editing tools for loss-of-function approaches. CRISPR-Cas9, TALENS and ZFNs introduce sequence- specific double-strand breaks in genomic DNA using different approaches for target sequence recognition. The DNA breaks induces error-prone endogenous DNA damage repair mechanisms (NHEJ: non-homologous end joining) that introduce frameshifting insertions and deletions. This subsequently leads to the complete knockout of a targeted gene. A second approach utilizes the homologous recombination pathway. For this purpose, a plasmid is used that contains sequences homologous to the flanking regions of the double-strand break and additionally any desired sequence that is subsequently integrated into the gene. This enables not only to silence genes by introducing frame-shifts but allows for introduction of base specific substitutions enabling reversion of mutations, introduction of mutations, alteration of promoter sequences (thus activation and inactivation of gene expression) and other applications.

mutation or amplification of one or more genes) and not according to their tissue origin (i.e. colon or lung cancer). This is usually a main consideration when designing target validation experiments. Knockdown of a specific target shall only exhibit the desired effects, e.g. reduction of proliferation and induction of apoptosis, in a subset of cell lines tested, namely those that display the specific oncogenomic alteration to be targeted. On the other hand, it is absolutely crucial that other cells, whether they are tumor cell lines or normal cells, do not respond to the knockdown of that particular target (Fig. 4). Only then a potential therapy is expected to have little negative side effects and a reasonable therapeutic window. For a comprehensive general review on the topic of how to select and validate successful drug targets from a pharmaceutical point of view the reader is referred to (Gashaw et al. 2011).

As compared to large-scale RNAi screening campaigns, where the focus often lies on screening as many genes as possible with a limited number of cell line models and read-out options, target validation approaches should be performed with the goal to verify the effects of addressing one gene in a number of different cell line models and using multiple readouts. This enables the researcher to confirm that the effects observed are really specific to a knockdown of the target and not due to off-target effects or cell line-specific artefacts.

In order to facilitate rational selection of the appropriate cell line models e.g. on the basis of mutation or amplification, several databases are publicly available, including the Catalogue Of Somatic Mutations In Cancer (COSMIC) (Forbes et al. 2010) or the TCGA Copy Number Portal of the BROAD Institute (Weinstein et al. 2013).

As each tumor cell line differs in mutation and amplification of a large variety of genes, it is difficult to discern which of these alterations are the (main) drivers of the tumor. Therefore, the panel of cell lines chosen for target validation approaches needs to be large enough to “dilute out” effects that might be contributed by other alterations a cell might have in addition to the selected alteration that is the basis of the stratification hypothesis. In general, we aim at ≥ 10 cell lines to cover both, the sensitive and insensitive status towards a given treatment.

To further increase confidence in the experimental observations, usually several siRNA of shRNA constructs are used to target one specific gene. Some researchers prefer to pool constructs while others rather test the constructs separately. There are pro and contra arguments to each approach and this has caused some controversy in the community (Smith 2006). We generally observe that pooling constructs increases the rate of false positives (see above) as off-target effects from several constructs contribute to the overall phenotype. We aim at identifying ≥ 5 siRNA or shRNA constructs per target that show greater than 90% reduction in mRNA levels. These are tested individually in phenotypic assays to validate the target.

After an early phase of euphoria about the potential of RNAi toward understanding gene functions, it soon became clear that RNAi has its pitfalls, very much like all screening technologies. Off-target effects are a severe problem of experimental results originating from RNAi. Unspecific off-target effects, such as

general toxicity of transfection or a possible interferon response triggered by foreign RNA in mammalian cells, can be easily identified by proper parallel experimental controls.

In contrast, sequence-based off-targets are a problem, especially for high-throughput RNAi screening, which requires special attention and thorough analysis of experimental data. Sequence-based off-target effects are understood as being caused by complementarity of the siRNA seed region (the 5'-proximal nt 2-8 of the siRNA guide or antisense strand) to the 3'-UTR sequence of unintended mRNA transcripts. This miRNA-like binding of siRNAs is a long-known and well-studied problem (Birmingham et al. 2006). However, only recently computational and experimental methods became available, which allow one to tackle the problem in large scale screens (Buehler et al. 2012).

Furthermore, experimental methods that allow the confirmation that a phenotype is caused by an on-target knockdown rather than an unintended off-target effect are available (Buehler et al. 2012). For example, in so-called C911 control siRNAs, nucleotides 9-11 are changed to their complement (e.g. 5'-CCG-3' to 5'-GGC-3') compared to the specific siRNA under investigation. The three mismatches are designed to prevent cleavage of the specific target mRNA, while still allowing miRNA-like effects *via* the seed region. Such C911 control siRNAs have become popular in helping to confirm whether phenotypes result from the knockdown of the intended target or are likely due to seed-based off-targeting.

A major issue arises, however, from the fact that some of the primary screening hits from large-scale screening campaigns may be false positives, and those have to be de-validated in subsequent validation campaigns which raises costs in both, time and money. It is thus very important to acknowledge and address potential pitfalls leading to false results in as many ways as possible in the planning and the setup of a screen. Such considerations include e.g. the choice of siRNA libraries. For increased specificity and favored incorporation of the siRNA guide strand into the RISC, vendors offer chemically modified siRNA libraries that are optimized in terms of thermodynamic siRNA duplex asymmetry, reduction of immunostimulatory sequence motifs, avoidance of miRNA-like off-target effects and other parameters (for more details, see article by Grünweller and Hartmann in this issue). Bioinformatics sequence analysis of the siRNAs before screening allows integration of most recent knowledge about changes in gene annotation of the human genome. Applying available bioinformatics analyses that are able to predict off-target effects in primary screening can significantly help to identify false positive results before validation and thus help reducing the costly de-validation of false positive results.

5. Do tools for genome editing represent alternatives to RNAi?

Genome editing tools offer novel opportunities for target validation and screening approaches (Fig. 6). In contrast to RNAi where an incomplete knockdown is achieved at the transcript level, gene editing technologies like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) or CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) disrupt the gene function by a complete knockout at the gene level [31]. This is achieved by utilizing endogenous NHEJ (non-homologous end joining) or HDR (homology-directed DNA repair) mechanisms after introduction of double strand breaks in the DNA. While ZFNs and TALENs are nucleotide sequence-specific peptide constructs fused to endonuclease domains that are difficult to use in high-throughput approaches, the advent of genome-wide CRISPR-Cas9 can be regarded as an alternative to RNAi. CRISPR-Cas9 is a fusion molecule that combines a synthetic guide RNA (instead of a peptide as for ZNFs and TALENs) fused to an endonuclease (Gaj et al. 2013). Although CRISPR-Cas9 is a promising novel tool in the repertoire of biotechnological methods for functional genomics, caution is advised as it was shown recently that also CRISPR-Cas9 constructs can suffer from unspecific binding (Kuscu et al. 2014). So far, the potential for off-targeting of gRNA-guided Cas9 constructs is insufficiently understood and thus needs more investigation. The flexibility in application and the availability of a large amount of specialized assays makes RNAi still a valuable tool. However, gene editing might turn out to be a complementary technique offering additional confidence in the confirmation of phenotypes at the molecular level.

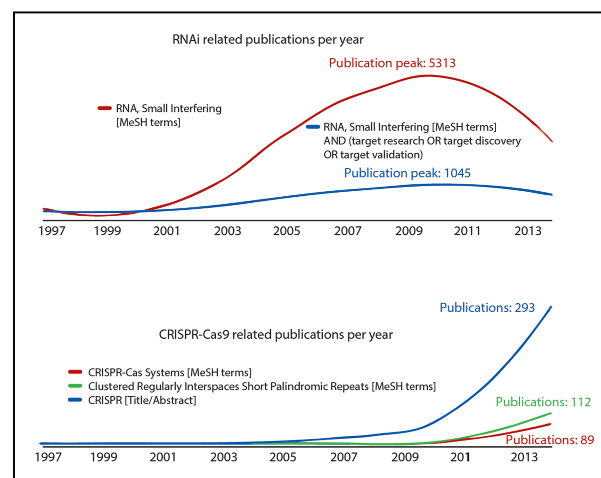

Fig. 7: Pubmed analysis results for publication trends. Note that the MESH terms "CRISPR-Cas Systems" and "Clustered Regularly Interspaced Short Palindromic Repeats" were introduced in 2013, respectively.

6. Conclusion and future perspectives

Since drug development has become more expensive and more difficult for various reasons, new strategies are required not only in terms of experimental setups but also at the level of business and collaborations. Sophisticated target validation approaches are available that enable an in-depth validation of putative drug targets which are about to enter the drug development pipeline. A successful target validation campaign should lead to earlier decisions on success or failure of a drug target, thereby reducing the overall attrition rate of the drug discovery process.

RNAi is a mature technique that enables effective screening and validation of targets for a large array of different phenotypes, with various assays at hand. The availability of complex assays for functional loss-of-function experiments makes novel target areas accessible that are difficult to approach by other methods. Although alternative methods have recently become available that may complement RNAi in the near future, loss-of-function screening by siRNAs and shRNAs remains an invaluable tool for deciphering gene functions associated with many disease-relevant phenotypes.

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