In this situation, new biologic molecules need to be generated de novo. However, this is a very time-consuming process. Therefore, one should consider whether validating upstream or downstream targets within the same pathway using existing tool compounds to achieve partial validation of the target and pathway of interest would be an acceptable alternative.

3.2 Genetic Approaches

Genetic approaches are complementary strategies for evaluating targets difficult for using biologics due to the lack of accessibility of targets or the availability of biologic tool compounds. There has been a growing collection of genetic approaches that investigators can choose from for validating drug targets. These methods include but are not limited to adeno-associated virus (AAV), lentivirus, RNA interference (RNAi), zinc-finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN), meganuclease, and CRISPR-Cas9 technologies.

Adeno-associated virus (AAV) can be used to knock out gene expression or to introduce genes in mice for in vivo target validation. AAV has been used to express a short hairpin RNA (shRNA) in the hypothalamus to generate a mouse model of obesity (Garza et al. 2008). AAV-induced knockout of vascular endothelial growth factor (VEGF) in the lung generated a model of emphysema (Tang et al. 2004). The introduction of genes by AAV in mice can also replace the use of therapeutic proteins for target validation. This can be achieved by introducing cDNAs into recombinant AAV vectors and injecting mice with rAAV. AAV represents a genetic approach for in vivo target validation. Similarly, lentiviral vectors have also been combined with RNAi approaches to achieve gene knock down for target validation (Rubinson et al. 2003; Ventura et al. 2004). However, it is important to note that gene disruption by RNAi approaches can be variable and short-lived. Therefore, phenotypic observations need to be interpreted with caution.

On the other hand, genome editing approaches such as ZFN (Urnov et al. 2010), TALEN (Sun and Zhao 2013), meganuclease (Menoret et al. 2013), and CRISPR-Cas9 (Platt et al. 2014) directly modify the DNA with nucleases to generate permanent modifications of the target genes within the genome for in vivo gene function validation. The overall concept of genome editing is to introduce a double-stranded break (DSB) within the target gene by directing the nuclease to the specific loci with complementary sequences. Following DSB, disruption of the gene or introduction of new gene sequence can be performed. There has been a rapid adoption of these technologies in the recent years. Among these technologies, CRISPR-Cas9 is considered to be a major advance for sequence-specific genome editing due to potential advantages of improved efficiency, ease of design, and potentially reduced off target editing over earlier genome editing approaches. Overall, gene editing approaches have a higher efficiency than generating knockout model by homologous recombination (Gaj et al. 2013). The advancement of these genetic techniques has offered new options for in vivo target validation.