International Journal of Scientific Multidisciplinary Research (IJSMR)
Vol.1, No.4, 2023: 312-330

Genetic Pharmacotherapy

Rehan Haider
Riggs Pharmaceutical Karachi, Department of Pharmacy, University of Karachi
Corresponding Author: Rehan Haider rehan_haider64@yahoo.com

A R T I C L E I N F O

Keywords: GPCRs, Computer-Aided Drug Discovery, Depression, Drug Resistance, Gene Polymorphism

Received : 2 March
Revised : 17 April
Accepted: 20 May

A B S T R A C T

In current drug development, proof-of-concept—determining whether a ligand engaging its target is likely to be therapeutic—requires specific ligands. This presents a catch-22, as the motivation to develop ligands requires proof-of-concept studies that cannot be conducted without ligands. A strategy we term genetic pharmacotherapy—a refinement of genetic blockade focused on drug-gable targets—obviates the catch-22 by enabling proof-of-concept studies before the development of specific ligands via genetic means in mouse models. In this strategy, which could help avert investment in molecular entities that will ultimately prove therapeutically efficacious, a gene is conditionally down-regulated via a molecular switch in adult mice. Both the precise temporal control of the intervention and the consequent change in the target protein function parallels the administration of drugs, with the additional advantage of perfect specificity. Moreover, genetic pharmacotherapy overcomes the impediment of the blood-brain barrier, which makes developing ligands for psychiatric disorders particularly challenging

DOI: https://doi.org/10.55927/ijsmr.v1i4.4374
E-ISSN: 2986-5042
https://journal.formosapublisher.org/index.php/ijsmr

INTRODUCTION

The conceptual foundation and requisite components of Genetic Pharmacotherapy We outline genetic pharmacotherapy as the use of a genetic intervention to achieve a pharmacological impact. Genetic pharmacotherapy has two necessities. First, the genetic blockade has to be standard — accomplishing all cells inside the frame, together with the mind to Simulate organism-extensive drug distribution. 2nd, induction of gene modulation needs to be temporally controllable, instead of originating throughout embryogenesis, so that focus on modulation occurs as it would with drug management. Even as conventional knockout strategies were used drastically to observe the jobs of proteins in an extensive range of Issues, constitutive mutations are regularly lethal in childhood precluding the having a look at target gene function in maturity (Lewandoski, 2001).{1} Furthermore, many non-deadly knockouts of genes of hobby elicit paradoxical phenotypes — phenotypes which can be contrary to the results of the pharmacologic blockade of the identical target in maturity — as a result of developmental compensations (Gingrich & Fowl, 2000){.2} The genetic approach that satisfies those requirements is a refinement of Cre-lox recombination, in which, in my opinion, silent mutations are brought. One mutation drives a ligand-inducible effector enzyme that enables target modulation, and the alternative mutation makes the target gene of interest susceptible to inactivation utilizing the effector enzyme. This includes breeding a mouse carrying the inducible effector — CreERT — with an animal wearing the effector-sensitized foxed gene of the hobby. In the ensuing progeny, inducing the CreERT produces irreversible goal modulation paralleling the institution of pharmacotherapy.Origins of the inducible-are method In Cre-lox recombination, Cre combines — from P1 bacteriophage — recognizes closely spaced 34-base pair loxP sequences, excises the intervening collection, and recombines the flanking strands. at the same time as positioned strategically using homologous recombination, the excision.

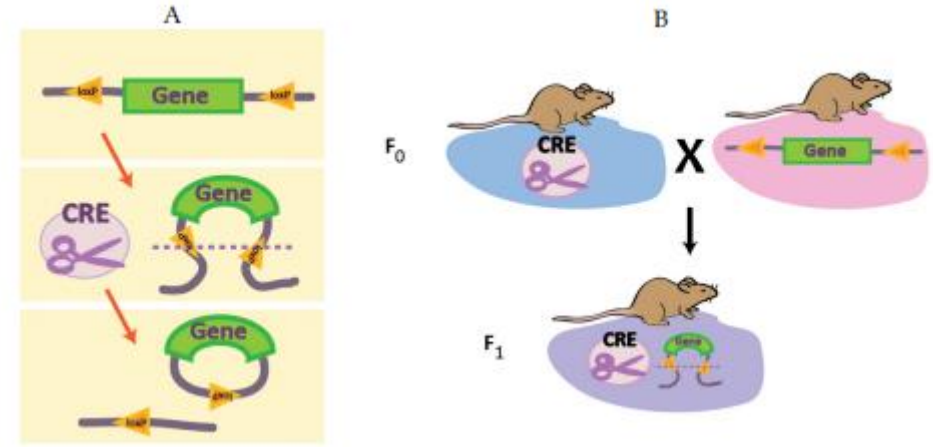

Figure 1. Removal of Targeted DNA Sequences by Cre-Lox Recombination

THEORETICAL FRAMEWORK

In 1992, groups (Lakso et al., 1992; Orban et al., 1992){5,6} said the number one use of Cre in mice to acquire tissue-precise expression. In 1994, Rajewsky and friends (Guet al.,1994){7} hired Cre as a conditional gene-targeted device to skip the pleiotropic embryonic lethality of the same null mutation. Tissue-specific Cre-mediated conditional gene inactivation has enabled investigators to address questions that might be in any other case intractable in global knockouts (Lewandoski, 2001). but, as cited, to superbly simulate remedy with a drug, which permeates the complete body, inactivation of the goal gene has to take area in every tissue kind — and for that reason, Cre expression needs to be driven with the useful resource of a universally expressed promoter together with the hybrid bird beta-actin promoter and cytomegalovirus enhancer (CAG) promoter. This promoter modified dramatically demonstrated to power the expression of better green fluorescent protein (EGFP) in sincerely every mobile kind to produce inexperienced mice (Okabe et al., 1997) {8{(Figure2).

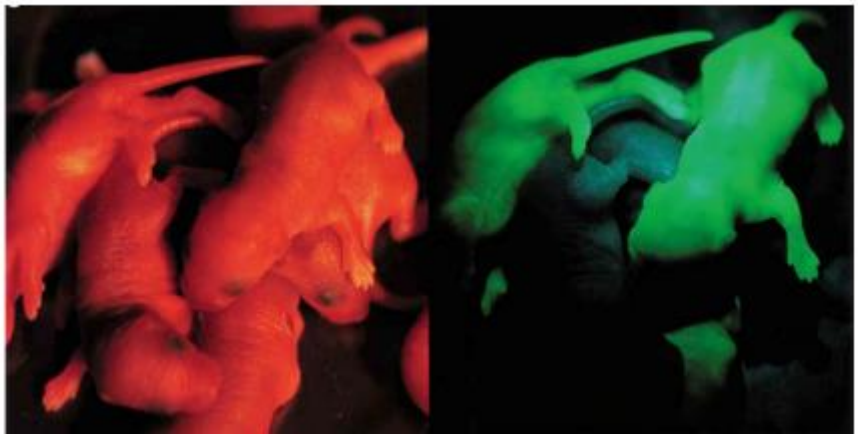

Figure 2. The Transgenic CAG Promoter Drives Expression Universally

Five mouse pups are seen under normal light (left); under blue light excitation (right), two of the pups fluoresce green as a result of expression of the ubiquitous CAG promoter that drives EGFP expression in all cell types (in the mice, only red blood cells and hair are not green) (From Okabe et al., 1997, with permission). Modified, ligand-activated CreERT enables temporal control over the target gene modulation.

To acquire temporal manipulation of Cre recombination, Chambon and co-workers (Feil et al., 1996; Metzger & Chambon, 2001; Metzger et al.,1995){ nine,10,11}created a ligand-established model of Cre, the chimeric protein CreERT, which mediates recombination most effective inside the presence of the drug tamoxifen or its derivatives. In CreERT, Cre is fused to the mutated ligand-binding the domain of the estrogen receptor, which recognizes tamoxifen and 4OH-tamoxifen, however no longer endogenous estrogen (discern 3a). As a steroid receptor of the nuclear receptor family, the Estrogen receptor of their inactive form is confined to the cytoplasm through affiliation with chaperone proteins (Giguère, 2003){12}. As with estrogen activation of the native steroid

receptor, tamoxifen releases CreERT from chaperone proteins, permitting the recombinase to diffuse into the nucleus to mediate web page-precise recombination. consequently, target gene inactivation is temporally managed and tamoxifen-established, as CreERT.An enhanced version, CreERT2, is now commonly used because it has approximately a 4-fold greater induction efficiency over CreERT (Indra et al., 1999; Lewandoski, 2001{thirteen,14} citations 112-115). For some transgene mixtures, there may be some recombination within the absence of tamoxifen (parent 4) (Hayashi, 2002){15}, so evaluation of the significance of pre-tamoxifen recombination will consequently be vital management. several ubiquitous inducible Cre lines are available (desk 1). An exchange strategy employs a modified progesterone receptor that is activated by way of RU486 (Kellendonk et al., 1999){16}

Table 1. Ubiquitously Expressed Inducible Cre Lines

Cre line	Strain name	Stock Number
CagCreERT1	B6.Cg-Tg(CAG-cre/Esr1*)5Amc/J	004682
RosaCreERT2	B6.129-Gt(ROSA)26Sortm1(cre/ERT2)Tyj/J	008463
UBC-CreERT2	B6.Cg-Tg(UBC-cre/ERT2)1Ejb/J	008085

Until recently, mice with floxed alleles were generated in individual laboratories and the range of commercially available lines of mice with floxed alleles was limited. Now, floxed mice are being made systematically through a multinational consortium, the Knockout Mouse Project (www.komp.org) (Skarnes et al., 2011){17}. The goal of KOMP is to produce conditional alleles of all expressed mouse genes. So far, 9,000 floxed alleles are or will soon be available, and floxed alleles for the remainder of mouse genes should be accessible in the near term. This comprehensive resource provides the basis for systematic target evaluation using genetic pharmacotherapy.

Comparability to target-specific drugs, with advantages and limitations Using inducible Cre-lox recombination to inhibit target gene expression offers comparability to, as well as many advantages over, ligand-based inhibition for proof-of-concept studies. Comparable to pharmacologic treatment, genetic pharmacotherapy enables control over the degree of target modulation, analogous to adjustments in drug dose, to assess dose response; and it also mimics the global, organism-wide action of drug intervention, which enables assessment of possible side effects due to pleiotropic target expression. The strategy's advantages include the preclusion of off-target effects via perfect target-specificity, and access to targets in the CNS via permeation of the blood-brain barrier, which stymies the evaluation of many drug candidates for CNS disorders. There are limitations; these include relative difficulty in targeting splice variants (protein isoforms from the same gene), as the strategy works at the DNA level, and an inability to capture the subtleties of drug action at targets that exhibit functional heterogeneity, i.e., differential structural conformations, drug affinities, and function of a single gene product. We examine each of these points in the further illustration below. Relative change induced in the target-protein function is commensurate to agonism and antagonism Often, to evoke a

response in the host cell system, a drug need to occupy only a fraction of the total receptors available—a function of both the drug's affinity and the intrinsic ability of the drug-receptor interaction to induce cellular change. This causes the dose-response curve to shift to the left of the receptor occupancy curve, so that a drug dose that elicits maximal tissue response may cause only partial occupation of the available receptors.

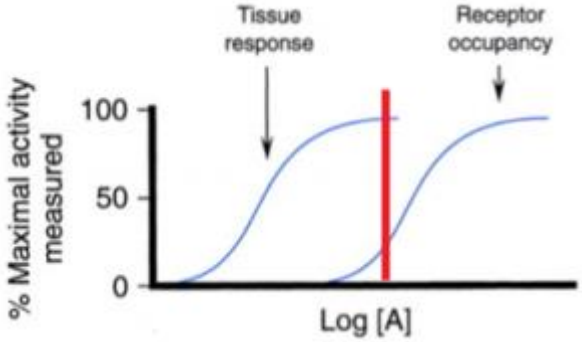

Figure 3. Maximal Tissue Response at Sub-Maximal Receptor Occupancy

Based on efficacy, drug doses generally need to activate only a fraction of the total available receptors to induce a maximal tissue response. In this illustration, the drug dose that induces maximal tissue response (red line) activates only a fraction of the total available receptors. (Modified from: Ross & Kenakin, 2001, with permission from McGraw-Hill Companies, Inc.) Example we focus mainly on its application to simulate target antagonism, as the majority of drugs are inhibitors (Copeland et al., 2007; Li et al., 2007){ 21,22}, and the accessibility of both ubiquitous CreER For example, opiate agonists etorphine and sufentanil have significant analgesic activity at very low receptor occupancy — approximately 2% at the ED50 (Rosenbaum et al., 1984);{18} and in another example, only 22% receptor occupancy is needed for half-maximal stimulation by PEG-TPOm, a mimetic peptide agonist in development for protection against chemotherapy-induced Thrombocytopenia (loss of blood platelets) (Samtani et al., 2009).{19} The antipsychotic dopamine D2 receptor antagonist olanzapine achieves optimal clinical efficacy at approximately 60% receptor occupancy (Mamo et al., 2007){20}. Similarly, genetic pharmacotherapy need not produce full target inhibition to elicit a response. Quantitative gradation in target protein expression (in analogy to a drug dose range) can be controlled by floxing one or both alleles of the gene of interest, and by tamoxifen dose and frequency of administration; in adult mice, tamoxifen is generally administered once daily for 5 days to achieve full recombination. Varying tamoxifen dosing can then mimic the range of drug action..While the Cre-lox strategy can also be used to simulate agonism, as discussed later, T2 mice and the floxed target of choice should now enable systematic in vivo target evaluation.

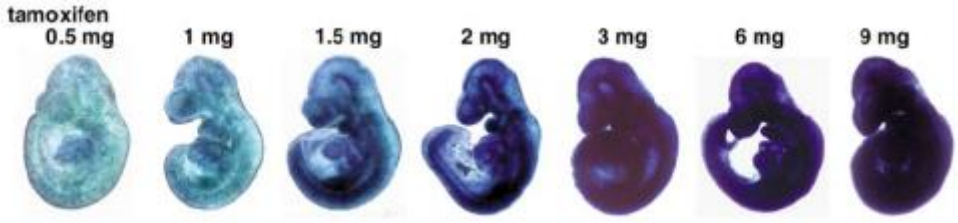

Figure 4. Tamoxifen Dose-Dependent Recombination in Embryos with CreERT

Driven by a universally expressed promoter. Although Cre-mediated gene modulation is irreversible, "dosing" as a percentage of cells undergoing recombination can still be controlled. Whole mount transgenic embryos were assayed 9.5 days post-coitum for the activity of the target gene, β-galactosidase, whose expression had been induced by tamoxifen at the indicated doses (per 40 kg mouse weight) 24 hours prior via intraperitoneal injection of the pregnant dam. (From: Hayashi, 2002, with permission).

Ubiquitously expressed effector simulates organism-wide pharmacologic actions. In this gene expression modulation system, the effector protein CreERT2 controls the expression of the target gene (for the candidate drug target). To achieve the closest simulation of a pharmacologic agent, which has organism-wide effects, it is paramount that a ubiquitously expressed locus such as Rosa26 (Soriano, 1999; Ventura et al., 2007) {23.24}be used to drive the effector protein expression (Table 1). This way it is possible to assess the phenotypic consequences of pleiotropy occurring in targets — that is, the side effects arising from inhibiting a gene product that participates in multiple signaling or metabolic pathways or different tissues (for further discussion of the relevance of pleiotropies to pharmacotherapy, Hodgkin1998; Searls, 2003){25,26}. While small interfering RNA (siRNA) has been used extensively for controlled gene inhibition in proof-of-concept studies, siRNA is not drug-like, as it must be delivered to the tissue of interest (Davidson & McCray, 2011) {27} Superiority over pharmacologic: preclusion of off-target effects via perfect.

Target-specificity Since Paul Ehrlich, drug design has aspired to the ideal of "magic bullet" drugs that seek out only "enemy targets" that are involved in pathology while leaving the body unharmed (Parascandola, 1981){28}. Lack of target specificity not only severely limits the use of small molecules as therapeutics — for example, the clinical use of anti-Parkinsonian drugs pergolide and cabergoline have been greatly limited because their off-target effects cause valvular heart disease (Kaiser et al., 2009) {29} — but also restricts their use as experimental tools in proof-of-concept evaluation (Peterson, 2008){30}. Although promiscuous targeting and multi-receptor activity do produce therapeutic benefits when single-receptor action does not — for example, in the case of stations and psychotropic medications (Kaiser et al., 2009; Peterson, 2008), it is nonetheless preferable that well-defined functional outcomes be understood through well-delineated biochemical actions.

Even a drug with a high specificity that achieves a desirable outcome in an animal model or clinically still cannot be concluded to work only via the pathway assumed. Genetic pharmacotherapy, on the other hand, enables the demonstration of precise, controlled causality in vivo studies. This is not to say

that the benefits of polypharmacy cannot be addressed with genetic pharmacotherapy; indeed, the synergy of inhibiting multiple targets simultaneously could be assessed in mice with multiple floxed alleles or by combining genetic pharmacotherapy with traditional pharmacotherapy. It may be impractical to translate findings from such an experiment into a ligand that accomplishes the same synergistic inhibition, but it would enable investigators to parse precisely which targets mediate the desired results and could guide drug optimization efforts.

Along these lines, genetic pharmacotherapy raises the possibility of validating previously established targets. For example, every antipsychotic drug approved so far has dopamine D2 receptor-blocking activity based on the presumed mode of action of first-generation antipsychotics, which was an entirely serendipitous discovery. However, their therapeutic efficacy likely involves interaction with other targets; for instance, antipsychotics inhibit KCNH2, a recently described potassium channel, found to be overexpressed in the brains of patients with schizophrenia (Huffaker et al., 2009){31}. The authors state: "Whereas D2 receptor affinity is thought to account for the therapeutic effects of antipsychotics, KCNH2 binding is responsible at least for side effects such as altered QT interval or even sudden cardiac failure. Given that KCNH2 controls neuronal excitability and firing patterns, could the therapeutic effects of antipsychotic drugs also, be related to their affinities for the brain-specific isoforms of KCNH2? (Huffaker et al., 2009)" With the present lack of specific KCNH2 ligands, genetic pharmacotherapy could enable further dissection of this possibility and resolve the conceptual justifications guiding the development of compounds with similarly intended actions, pharmacologic or therapeutic Specific advantages of CNS targets Genetic pharmacotherapy offers particular advantages for proof-of-concept studies for CNS disorders. Such studies face not only the obstacle of designing high-affinity, high-specificity ligands, as in the evaluation of targets in other tissues but also the blood-brain barrier — the superfine filter composed of endothelial cells lining the brain capillaries and astrocytes — that either block or actively transports out more than 98% of candidate drugs (Miller, 2002).{32}

Genetic pharmacotherapy circumvents this obstacle because tamoxifen permeates the blood-brain barrier freely. Limitations of achieving target inhibition via DNA modification While drugs can distinguish between different protein isoforms arising from RNA splice variants (Thompson et al., 2011){33} that may have different anatomical and functional specificities (Huffaker et al., 2009), genetic pharmacotherapy can not make such a distinction easily, as goal inhibition is mediated via modifications on the DNA level. Similarly, a drug may additionally engage differentially with receptors displaying practical heterogeneity — e.g., a receptor with awesome allosteric conformations and signaling complexes, varying using anatomical distribution (Mailman, 2007; Mailman & Murthy, 2010) {34,35}— a subtlety much less amenable to simulation with genetic pharmacotherapy but potential in a few cases with pharmacology.

The D2 dopamine receptor offers a conventional instance: agonist stimulation of presynaptic D2 dopamine auto receptor diminishes dopamine

synthesis and release, which may additionally obtain a dopamine antagonist-like effect submit-synaptically thru decreased dopamine neuro transmission — while preferential agonism of put up-synaptic D2 dopamine receptors would acquire the alternative effect. Selective focus on wonderful cell-type D2 receptors can not be done with a typical CreERT line, but the D2 receptor partial agonist omeprazole seems to show off such purposeful selectivity. although, a judiciously chosen tissue-precise inducible CreERT2 strategy may additionally permit such issues to be addressed with better precision than with pharmacology. For example, presynaptic dopamine D2 receptors have been selectively targeted with the use of a DAT-Cre motive force (Bello et al., 2011){36}, and DAT-CreERT2 mice were suggested (Engblom et al., 2008){37}, so such adjustments ought to be brought on in maturity to version an autoreceptor-selective dopamine D2 receptor antagonist.

Evolution of Genetic Pharmacotherapy

Genetic pharmacotherapy builds on molecular developments of the last two decades. Below, we describe seminal applications of aspects of the genetic pharmacotherapy strategy, and, in certain instances, the molecular entities with actions mirroring the corresponding genetic intervention. Where we wish to build on these foundations is in illustrating the feasibility and advantages of applying this concept — specifically, by employing inducible Cre-lox technology — to evaluate new potential therapeutic drug targets systematically, particularly in the CNS, a possibility that has not yet been articulated until now.

Genetic Blockade in Lieu or Absence of Pharmacologic Blockade

The potential advantages of using genetic blockade in place of a pharmacologic blockade for CNS studies were described in 1996 in studies reporting the first transgenic expression of Cre in the mouse nervous system (Tsien et al., 1996):{38} "Studies of … mechanisms underlying vertebrate animal [behavior] have Traditionally been carried out using pharmacological blockade gene knockouts Provide an alternative means. While the two methods are complementary and genetic deletion is generally superior to pharmacological blockade concerning molecular and anatomical specificities and animal-to-animal reproducibility. For instance, while many antagonists cannot distinguish receptor isoforms. Genetic blockades can make that distinction. Likewise, a genetic blockade can be highly confined reproducible." The juxtaposition of the genetic and pharmacologic blockade, in this instance, pertains to their relative merits as experimental tools for addressing. Questions regarding memory formation as opposed to new therapeutic targets. In a related, concurrent study, the tetracycline trans-activator system was used for regional and temporal control over calcium-calmodulin-dependent kinase II (CamKII) expression to demonstrate the requirement of CamKII for both implicit and explicit memory formation (Mayford et al., 1996){39}

Tetracycline-regular table gene expression The tetracycline trans-activator system (Gossen & Bujard, 1992){40} offers another ligand-controlled gene expression system, in which the targeted gene is turned off or on by the administration of tetracycline or (more commonly) doxycycline (Figure 7a). In this system, the E. coli-derived tetracycline-controlled trans-activator (tTA)

drives target-gene transcription by binding to a modified Tet operator (tetO) sequence and this activity can be:

Diminished and switched off depending on varying concentrations of doxycycline. This strategy is adapted from the E. coli tetracycline-resistance operon, in which transcription of tetracycline resistance-mediating genes is negatively regulated by the tetracycline repressor (tetR). The presence of tetracycline causes the dissociation of tetR from the promoter region of the operon and enables the transcription of resistance genes. In contrast, as just mentioned, the modified trans-activator tTA, based on tetR, stimulates (as opposed to repressing) transcription when bound to minimal promoters, fused to tetO sequences, and the presence of low concentrations of tetracycline (<100 nM) or doxycycline prevents the binding of tTA to the tetO sequences and thereby halts transcription. In the modified Tet-on version of this system (Kistner et al., 1996){41}, doxycycline turns on target gene expression via a reverse tTA (rtTA). In this case, rtTA will bind tetO to activate transcription only in the presence of doxycycline.

While the Tet-regulate able strategy is appealing for the reversibility of target modulation (with the addition or removal of doxycycline administration) in inhibition studies, it is impossible to produce a silent mutation in the target gene, such as the introduction of loxP sites. To control the gene of interest, the tetO promoter must be substituted for the native promoter on one allele of the target gene, and the native promoter on the other allele, in turn, used to drive the. A study employed this strategy to examine the role of the dopamine transporter (DAT) in scaling learning (Cagniard et al., 2006).{42} In this tandem design, tissue specificity of target gene expression is maintained, but pre-doxycycline expression levels vary considerably; this is problematic for genetic pharmacotherapy because target gene function should be at basal levels before induction. In the strategy, the mutant mice expressed modestly reduced levels of DAT and it took several weeks of doxycycline administration to achieve significant suppression of DAT expression (Cagniard et al., 2006).{45} In another study examining the role of potassium SK3 channels, SK3 expression was several-fold higher than control and was suppressed with doxycycline (Bond et al., 2000){.43}

Though less ideal for systematic target evaluation when compared to CreERT2, the tetracycline-trans activator system has nevertheless been particularly powerful in modeling a disease state and subsequently assaying the proof-of-concept of its reversibility via doxycycline administration. In a landmark paper, Yamamoto and colleagues (2000) {44} created a mouse model of Huntington's disease (HD) by tetO-driven, stratum-restricted expression of a pathogenic version of the Huntington protein. By 4 weeks of age, the mice began to exhibit choreic movements and dystonia, and by 8 weeks showed striatal Huntington aggregates both hallmarks of HD. HD is progressive, without specific treatment or cure, and before this study was assumed to be inexorable in its course. However, abolishing the expression of mutant.

Huntington, by doxycycline administration in symptomatic mice, not only halted but also reversed the accumulation of protein aggregates and progressive

motor decline. Although developing a pharmacologic intervention for HD has yet to be achieved, this study demonstrated that blocking the expression of pathogenic Huntington in symptomatic subjects reversed manifestations of the disease and, indeed, could be viewed as having achieved a cure. This proof-of-concept motivates a search for drugs that would reverse or prevent Huntington's protein aggregate formation.

Kellendonk and colleagues (2006){45} used the tetracycline-transactivator system to address the possibility of reversing schizophrenia-like abnormalities induced by dopamine D2 receptor overexpression (D2OE) in the striatum. Imaging studies have shown that dopamine transmission is increased in patients with schizophrenia, involving both increased dopamine release and increased dopamine D2 receptor binding (Guillin et al., 2007){46}. The D2OE mice, in which the drove striatally restricted expression of an extra human D2R allele, exhibited elevated receptor binding capacity — 15% higher than control littermates. The pivotal finding was that dopamine dysfunction — previously thought only to account for the positive.

Symptoms, such as hallucinations and delusions, could be causally linked to cognitive deficits (Simpson et al., 2010).{47} D2OE mice showed altered dopamine transmission in the prefrontal cortex as well as selective cognitive impairment in working memory tasks, a prefrontal cortex-dependent process, without more general cognitive deficits — similar to cognitive impairments in patients with schizophrenia. Administering doxycycline to reverse the D2OE did not reverse the working memory impairment, suggesting that the cognitive deficits in these mice arose not from continued D2OE but as a consequence of D2OE during development. Whether D2OE occurs before the onset of schizophrenia in patients is not known; however, dopamine release is increased (Howes et al., 2008),{48} so there is clear evidence for increased dopamine transmission before the onset of schizophrenic symptoms. The earlier.

The consequences of increased dopamine transmission in the D2OE mice are consistent with the well-recognized inability of dopamine D2 receptor antagonists to ameliorate cognitive impairments. It must also be noted that while genetic blockade of transgene expression accomplished down-regulation of D2 receptor overexpression, it did not mimic actual D2 receptor antagonist pharmacotherapy fully, as doxycycline inhibited only the transgenic D2 receptors while D2 antagonists would block both the transgenic and native D2 receptors. In these studies, the tetracycline-transactivator system was used to model disease, and subsequently, doxycycline administration was used to turn off pathogenic protein expression to establish causality between protein and disease. In genetic pharmacotherapy studies, the introduction of an extra Tet-regular table allele could be used to test the effects of increasing target expression, mimicking the actions of a targeted agonist. One benefit of this strategy, as mentioned, is the reversibility of the target modulation; however, the transgenic mice would need to be engineered on a per-target basis, as there is no repository analogous to KOMP for tet-regular table alleles. Using the Tet-off system in inhibition studies, as in the DAT study, disadvantageously requires the engineering of two transgenic mice (for both the tetO and tTA alleles).

Genetic Blockade Techniques Beyond Inducible Cre and tTA

Genetic blockade as a proof-of-concept tool has also been used in several oncogenic signaling pathway studies. Although the blockade in the following two examples was accomplished via methods difficult to translate to other druggable targets, the studies successfully established a therapeutic proof-of-concept via genetic means in the absence of a high-specificity ligand against the endogenous target In one study, a silent mutation engineered into an oncogenic kinase allowed for its selective inhibition, whereas kinase inhibitor non-specificity had previously deterred such an investigation (Fan et al., 2002){49}. In a second study, tumorigenic expression of an endocrine receptor was suppressed by a truncated version of the same receptor (delivered via an adenovirus) that acted as a dominant-negative inhibitor (Min et al., 2003).{50} In both cases, the subsequently developed pharmacologic inhibitors produced the same effect as the genetic blockade, corroborating the parallel between genetic and pharmacologic target blockade.

Engineering a kinase for selective inhibition to determine its role in oncogenic signaling to overcome the lack of specific kinase inhibitors, Fan and colleagues engineered a silent mutation into a target kinase gene that allowed selective inhibition while retaining kinase activity before administration of the mutant kinase-specific antagonist, NaPP1 (Fan et al., 2002). Mice subcutaneously injected with cancer cells containing endogenous or NaPP1-sensitized versions of the epithelial growth factor receptor (EGFP) oncogene v-erbB grew tumors of similar size and with similar latencies — but the inhibitor NaPP1 suppressed growth only in tumors with the sensitized allele Fan and colleagues (2002) concluded that selective inhibitors of EGFR may effectively arrest cancer cell proliferation at a favorable therapeutic index, as basal signaling in normal cells is unlikely to be affected. Although inhibitors selective to the ErbB EGFR were not available at the time of this chemical-genetic blockade study, genetically engineering inhibitor sensitivity demonstrated the target's proof-of-concept. This incentivized further efforts to develop ligands specific to EGFR subtypes, such as ErbB. Indeed, initial marketing of Gefitinib, an EGFR tyrosine kinase inhibitor, was approved in 2003 for patients with non-small cell lung cancer, and Relations, another EGFR tyrosine kinase inhibitor, was approved for the same indication in 2004 and pancreatic cancer in 2005 (National Cancer Institute Online Drug Information, Pao, 2005){51}.

METHODOLOGY

The materials and methods used for genetic pharmacotherapy involve the use of transgenic mice expressing a molecular switch that allows for conditional down regulation of a target gene in specific tissues or cells. The switch can be activated by:the administration of an external inducer, which triggers the transcription of a smallRNA molecule that specifically targets the mRNA of the gene of interest, leading toits degradation.

To implement genetic pharmacotherapy, transgenic mice expressing the molecular switch are generated through the use of gene targeting technologies such as homologous recombination or CRISPR/Cas9-mediated gene editing.

These mice are then crossed with mice carrying a tissue-specific promoter to restrict the expression of the switch to a particular cell type or tissue.

To test the efficacy of genetic pharmacotherapy in a particular disease model, the transgenic mice are treated with the external inducer, and the resulting changes in the target protein function are assessed. This can involve a range of techniques, including Western blotting, immunohistochemistry, behavioral assays, and transcriptomic analysis.

RESULTS

The results of genetic pharmacotherapy have shown that this strategy can be a powerful tool for validating drug targets and identifying potential therapeutic interventions. By allowing for the conditional down-regulation of a target gene, genetic pharmacotherapy can demonstrate the role of a particular protein in a disease model and assess the potential therapeutic benefit of targeting that protein.In addition, genetic pharmacotherapy overcomes the limitations of traditional pharmacological approaches, such as the lack of specificity and difficulty in crossing the blood-brain barrier. By selectively targeting a specific gene in a particular tissue or cell type, genetic pharmacotherapy can achieve high specificity, and by delivering the inducer systemically, it can bypass the blood-brain barrier.

DISCUSSION

Genetic pharmacotherapy has several advantages over traditional drug development approaches and other gene expression modification methods. It allows for the validation of drug targets before the development of specific ligands, which can save time and resources, and it provides perfect specificity and temporal control of the intervention.However, genetic pharmacotherapy also has some limitations. It requires the generation of transgenic mice, which can be time-consuming and expensive, and it may not apply to all disease models. In addition, the off-target effects of the molecular switch and inducer system need to be carefully assessed.Despite these limitations, genetic pharmacotherapy has shown promise as a tool for drug discovery and development, particularly in the field of CNS disorders. With further development and refinement, this strategy has the potential to contribute to the development of new, innovative molecular therapies.

CONCLUSIONS

The genetic pharmacotherapy strategy enables testing the therapeutic proof-of-idea of goal modulation in the absence of particular ligands. It allows circumvention of the evidence-of-concept seize-22 in drug development, where the hazard of investing objectives with constrained validation often impedes the pursuit of particular ligands that would ultimately be demonstrated. It additionally minimizes the complementary problem of investment in selective ligands that would in the long run fail in scientific proof-of-idea studies (figure 14). The recently announced conditional allele library useful resource (Skarnes et al., 2011) will facilitate efforts to test multiple objectives with the inducible Cre strategy. Druggable targets that are in all likelihood to be disease-modifying have now been diagnosed (Hajduk et al., 2005; Knox et al., 2011; Zhu et al., 2010).52,53,54

The idea and tools of genetic pharmacotherapy were nicely mounted for a while, but their capacity for systematic application to evidence-of-concept assessment for drug improvement efforts has now not been fully appreciated. Genetic pharmacotherapy needs to prove to be an effective orthogonal tool (Hardy & Peet, 2004) for drug development. the dearth of funding in without a doubt progressive psychiatric tablets during the last many years underpins the tremendous unmet want for better remedies, because the burden of psychiatric contamination stays excessive and current remedies continue to be ineffective or nonexistent (Brundtland, 2001; Miller, 2010){55}. Critics of psychiatric drug development argue that because the etiology of fundamental intellectual ailments stays so poorly understood, adequate remedies can not be developed (Conn & Roth, 2008){56}. however, applying genetic pharmacotherapy in an increasing number of state-of-the-art mouse models of psychiatric problems promises to make the whole mouse genome on hand for drug discovery and so increase significantly the accessibility of molecular objectives for pharmacotherapies. We accept as true that this strategy will expedite the development of innovative new molecular healing procedures, in particular for CNS issues.

ACKNOWLEDGMENT

The crowning glory of this study venture could not be possible without the contributions and help of many human beings and corporations. we're deeply grateful to all people who played a function in the success of this undertaking I would like to thank My Mentor [Dr. Naweed Imam Syed Prof Department of Cellular Biology at the University of Calgary for his or her beneficial enter and steerage in some unspecified time in the future of the studies gadget. Their insights and knowledge had been instrumental in shaping the path of this venture.

Authors' Contribution I would like to grow our sincere manner to all the individuals on our check, who generously shared their time, studies, and insights with us. Their willingness to engage with our studies has become important to the success of this assignment, and we're deeply grateful for her participation.

REFERENCES

Lewandoski, M. (2001) Conditional control of gene expression in the mouse. Nature Rev Genet, Vol. 2, No. 10, pp. 743-755

Gingrich, J.A. and Hen, R. (2000) The broken mouse: the role of development, plasticity, and environment in the interpretation of phenotypic changes in knockout mice. Curr. Opin. Neurobiol., Vol. 10, No. 1, pp. 146-152

Nagy, A. (2000) Cre recombinase: the universal reagent for genome tailoring. Genesis, Vol. 26, No. 2, pp. 99-109

Sternberg, N. and Hamilton, D. (1981) Bacteriophage P1 site-specific recombination. I. Recombination between loxP sites. J. Mol. Biol., Vol. 150, No. 4, pp. 467-486

Lakso, M., Sauer, B., Mosinger, B., Jr., Lee, E.J., Manning, R.W., YU, S.H., Mulder, K.L. and Westphal, H. (1992) Targeted oncogene activation by site-specific recombination in transgenic mice. PNAS, Vol. 89, No. 14, pp. 6232-6236

Orban, P.C., Chui, D. and Marth, J.D. (1992) Tissue- and site-specific DNA recombination in transgenic mice. PNAS, Vol. 89, No. 15, pp. 6861-6865

GU, H., Marth, J.D., Orban, P.C., Mossman, H. and Rajewsky, K. (1994) Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. Science, Vol. 265, No. 5168, pp. 103-106

Okabe, M., Ikawa, M., Kominami, K., Nakanishi, T. and Nishimura, Y. (1997) 'Green mice' as a source of ubiquitous green cells. FEBS Lett., Vol. 407, No. 3, pp. 313-319

Feil R., Brocard J., Marquez B., LeMeur, M., Metzger D. and Chambon P. (1996) Ligand activated site-specific recombination in mice. PNAS, Vol. 93, No. 20, pp. 10887- 10890

Metzger, D. and Chambon, P. (2001) Site- and time-specific gene targeting in the mouse. Methods, Vol. 24, No. 1, pp. 71-80

Metzger, D., Clifford, J., Chiba, H. and Chambon, P. (1995) Conditional site-specific

Recombinase. PNAS, Vol. 92, No. 15, pp. 6991-6995

Giguère, V. (2003) In Bradshaw, R. A. and Dennis, E. A. (eds.), Handbook of Cell Signaling, Volume 3. Academic Press, Amsterdam, Vol. 3, pp. 35-38.

Indra, A.K., Warot, X., Brocard, J., Bornert, J.M., Xiao, J.H., Chambon, P. and Metzger, D. (1999) temporally controlled site-specific mutagenesis in the basal layer of the epidermis:

Comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and CreER(T2) recombinases. Nucleic Acids Res., Vol. 27, No. 22, pp. 4324-4327

Lewandoski, M. (2001) Conditional control of gene expression in the mouse. Nature Rev Genet, Vol. 2, No. 10, pp. 743-755

Hayashi, S. (2002) Efficient Recombination in Diverse Tissues by a Tamoxifen-Inducible Form of Cre: A Tool for Temporally Regulated Gene Activation/Inactivation in the Mouse. Dev. Biol., Vol. 244, No. 2, pp. 305-318

Kellendonk, C., Tronche, F., Casanova, E., Anlag, K., Opherk, C. and Schutz, G. (1999) Inducible site-specific recombination in the brain. J. Mol. Biol., Vol. 285, No. 1, pp. 175-182

Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., Jackson, D., Severin, J., Biggs, P., Fu, J., Nefedov,

M., de Jong, P.J., Stewart, A.F. and Bradley, A. (2011) A conditional knockout Resource for the genome-wide study of mouse gene function. Nature, Vol. 474, No.

7351, pp. 337-34217

Rosenbaum, J.S., Holford, N.H. and Sadée, W. (1984) Opiate receptor binding-effect relationship: sufentanil and etorphine produce analgesia at the mu-site with low fractional receptor occupancy. Brain Res., Vol. 291, No. 2, pp. 317-324

Samtani, M.N., Perez-Ruixo, J.J., Brown, K.H., Cerneus, D. and Molloy, C.J. (2009)

Pharmacokinetics and pharmacodynamics modeling of pegylated thrombopoietin

mimetic peptide (PEG-TPOm) after single intravenous dose administration in

healthy subjects. J Clin Pharmacol, Vol. 49, No. 3, pp. 336-350

Mamo, D., Kapur, S., Keshavan, M., Laruelle, M., Taylor, C.C., Kothare, P.A., Barsoum, P. and McDonnell, D. (2007) D2 Receptor Occupancy of Olanzapine Pamoate Depot Using Positron Emission Tomography: An Open-label Study in Patients with Schizophrenia. Neuro psychopharmacology, Vol. 33, No. 2, pp. 298-304

Copeland, R.A., Harpel, M.R. and Tummino, P.J. (2007) Targeting enzyme inhibitors in drug discovery. Expert Opin Ther Targets, Vol. 11, No. 7, pp. 967-97

Li, Q.-X., Tan, P., Ke, N. and Wong-Staal, F. (2007) Ribozyme technology for cancer gene target identification and validation. Adv. Cancer Res., Vol. 96, pp. 103-143

Soriano, P. (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet., Vol. 21, No. 1, pp. 70-71

Ventura, A., Kirsch, D.G., McLaughlin, M.E., Tuveson, D.A., Grimm, J., Lintault, L.,

Newman, J., Reczek, E.E., Weissleder, R. and Jacks, T. (2007) Restoration of p53

Function leads to tumor regression in vivo. Nature, Vol. 445, No. 7128, pp. 661-665

Hodgkin, J. (1998) Seven types of pleiotropy. Int. J. Dev. Biol., Vol. 42, No. 3, pp. 501-505

Searls, D.B. (2003) Pharmacophylogenomics: genes, evolution, and drug targets. Nat Rev Drug Discov, Vol. 2, No. 8, pp. 613-623

Davidson, B.L. and McCray, P.B. (2011) Current prospects for RNA interference-based therapies. Nature Publishing Group, Vol. 12, No. 5, pp. 329-340.

Parascandola, J. (1981) The theoretical basis of Paul Ehrlich's chemotherapy.

Keiser, M.J., Setola, V., Irwin, J.J., Laggner, C., Abbas, A.I., Hufeisen, S.J., Jensen, N.H., Kuijer, M.B., Matos, R.C., Tran, T.B., Whaley, R., Glennon, R.A., Hert,

J., Thomas, K.L.H., Edwards, D.D., Shoichet, B.K. and Roth, B.L. (2009) Predicting new Molecular targets for known drugs. Nature, Vol. 462, No. 7270, pp. 175-181.

Peterson, R.T. (2008) Chemical biology and the limits of reductionism. Nat. Chem. Biol., Vol. 4, No. 11, pp. 635-638

Huffaker, S.J., Chen, J., Nicodemus, K.K., Sambataro, F., Yang, F., Mattay, V., Lipska, B.K., Hyde, Song, J., Rujescu, D., Giegling, I., Mayilyan, K., Proust, M.J., Soghoyan, A., Caforio, G., Callicott, J.H., Bertolino, A., Meyer-Lindenberg, A., Chang, J., Ji, Y., Egan, M.F., Goldberg, T.E., Klein man, J.E., Lu, B. and Weinberger, D.R. (2009) A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat. Med., Vol. 15, No. 5, pp. 509-518 32Miller, G. (2002), Science, Vol. 297, pp. 1116-1118.

Thompson, C.H., Kahlig, K.M. and George, A.L. (2011) SCN1A splice variants exhibit

divergent sensitivity to commonly used antiepileptic drugs. Epilepsia, Vol. 52, No. 5,

pp. 1000-1009

Mailman. (2007) GPCR functional selectivity has a therapeutic impact. Trends Pharmacol. Sci., Vol. 28, No. 8, pp. 390-396

Mailman, R.B., and Murthy, V. (2010) Ligand functional selectivity discovery. Neuropsychopharmacology,

Vol. 35, No. 1, pp. 345-346

Bello, E.P., Mateo, Y., Gelman, D.M., Noaín, D., Shin, J.H., Low, M.J., Alvarez, V.A.,

Lovinger, D.M. and Rubinstein, M. (2011) Cocaine super sensitivity and enhanced

Motivation for reward in mice lacking dopamine D2 auto receptors. Nat. Neurosci.,

Vol. 14, No. 8, pp. 1033-1038

Engblom, D., Bilbao, A., Sanchis-Segura, C., Dahan, L., Perreau-Lenz, S., Balland, B.,

Parkitna, J.R., Luján, R., Halbout, B., Mameli, M., Parlato, R., Sprengel, R., Lüscher,

C., Schütz, G. and Spanagel, R. (2008) Glutamate receptors on dopamine neurons

control the persistence of cocaine seeking. Neuron, Vol. 59, No. 3, pp. 497-508

Tsien, J.Z., Chen, D.F., Gerber, D., Tom, C., Mercer, E.H., Anderson, D.J., Mayford, M., Kandel, E.R. and Tonegawa, S. (1996) Sub-region- and cell type-restricted gene

knockout in mouse brain. Cell, Vol. 87, No. 7, pp. 1317-1326

Mayford, M., Bach, M.E., Huang, Y.Y., Wang, L., Hawkins, R.D. and Kandel, E.R. (1996) Control of memory formation through regulated expression of a CaMKII transgene. Science, Vol. 274, No. 5293, pp. 1678-1683

Gossen, M. and Bujard, H. (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. PNAS, Vol. 89, No. 12, pp. 5547-5551

Kistner, A., Gossen, M., Zimmermann, F., Jerecic, J., Ullmer, C., Lübbert, H. and Bujard, H. (1996) Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. PNAS, Vol. 93, No. 20, pp. 10933-10938

Cagniard, B., Beeler, J.A., Britt, J.P., McGeehan, D.S., Marinelli, M. and Zhuang, X. (2006) Dopamine scales performance in the absence of new learning. Neuron, Vol. 51, No. 5, pp. 541-547

Bond, C.T., Sprengel, R., Bissonnette, J.M., Kaufmann, W.A., Pribnow, D., Neelands, T., Storck, T., Baetscher, M., Jerecic, J., Maylie, J., Knaus, H.G., Seeburg, P.H. and Adelman, J.P. (2000) Respiration and parturition affected by conditional overexpression of the Ca2+-activated K+ channel subunit, SK3. Science, Vol. 289, No. 5486, pp. 1942-1946

Yamamoto, A., Lucas, J.J. and Hen, R. (2000) Reversal of neuro pathology and motor dysfunction in a conditional model of Huntington's disease. Cell, Vol. 101, No. 1, pp. 57-66

Kellendonk, C., Simpson, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H and Kandel, E.R. (2006) Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. Neuron, Vol. 49, No. 4, pp. 603-615

Guillin, O., Abi-Dargham, A. and Laruelle, M. (2007) Neurobiology of dopamine in

schizophrenia. Int. Rev. Neurobiol., Vol. 78, pp. 1-39

Simpson, E.H., Kellendonk, C. and Kandel, E. (2010) A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron, Vol. 65, No. 5, pp. 585-59

Howes, O., Montgomery, A., Valli, I., Asselin, M., Murray, R., Grasby, P. and Mcguire, P. (2008) Striatal dopamine dysfunction predates the onset of schizophrenia and is linked to prodromal symptoms and neurocognitive function. Schizophr. Res., Vol. 102, No. 1-3, pp. 30-30

Fan, Q.-W., Zhang, C., Shokat, K.M. and Weiss, W.A. (2002) Chemical genetic blockade of transformation reveals dependence on aberrant oncogenic signaling. Curr. Biol., Vol. 12, No. 16, pp. 1386-1394

Min, Y., Adachi, Y., Yamamoto, H., Ito, H., Itoh, F., Lee, C.-T., Nadaf, S., Carbone, D.P. and Imai, K. (2003) Genetic blockade of the insulin-like growth Factor-I receptor: a promising strategy for human pancreatic cancer. Cancer Res., Vol. 63, No. 19, pp. 6432-6441

Pao, W. (2005) Epidermal Growth Factor Receptor Mutations, Small-Molecule Kinase Inhibitors, and Non-Small-Cell Lung Cancer: Current Knowledge and Future

Directions. J. Clin. Oncol., Vol. 23, No. 11, pp. 2556-2568

Hajduk, P.J., Huth, J.R. and Tse, C. (2005) Predicting protein druggability. Drug Discovery Today, Vol. 10, No. 23-24, pp. 1675-1682

Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V., Djoumbou, Y., Eisner, R., Guo, A.C. and Wishart, D.S. (2011) DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. Nucleic Acids Res, Vol. 39, Database issue, pp. D1035-1041

Zhu, F., Han, B., Kumar, P., Liu, X., Ma, X., Wei, X., Huang, L., Guo, Y., Han, L., Zheng, C. and Chen, Y. (2010) Update of TTD: Therapeutic Target Database. Nucleic Acids Res, Vol. 38, No. Database issue, pp. D787-791

Brundtland, G.H. (2001) From the World Health Organization. Mental health: new

understanding, new hope. JAMA, Vol. 286, No. 19, pp. 2391

Conn, P.J., and Roth, B.L. (2008) Opportunities and Challenges of Psychiatric Drug

Discovery: Roles for Scientists in Academic, Industry, and Government Settings. Neuropsychopharmacology, Vol. 33, No. 9, pp. 2048-2060