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Review Article

ANIMAL MODELS FOR HUNTINGTON'S DISEASES: A REVIEW

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ABSTRACT

Huntington's disease (HD) is an inherited autosomal, progressive neurodegenerative disorder associated with involuntary abnormal movements (chorea), cognitive impairments and psychiatric disturbances. HD is caused by an abnormal expansion of a CAG region located in exon 1 of the gene encoding the huntingtin protein (Htt) and is the causative factor in the pathogenesis of HD Animal models of HD have provided insight into disease pathology and the outcomes of thera- peutic strategies. Earlier studies of HD most often used toxin-induced models to study mitochondrial impairment and excitotoxicity-induced cell death, which are both mechanisms of degeneration seen in the HD brain. These models, based on 3-nitropropionic acid and quinolinic acid, respectively, are still often used in HD studies. The discovery in 1993 of the huntingtin mutation led to the creation of newer models that incorporate a similar genetic defect. These models, which include transgenic and knock-in rodents, are more representative of the HD progression and pathology. An even more recent model that uses a ovine transgenic model (sheep model).fly models ,cell cultures models for better understanding of gene mutation in and in mammalian and nonhuman primates, as it is difficult to produce genetic models in these species. This article examines the aforementioned models and describes their use in HD research, including aspects of the creation, de- livery, pathology, and tested therapies for each model.

Key Words: 3-nitropropionic acid; animal models; Huntington's disease; knock-in mice; quinolinic acid; transgenic rodents.

INTRODUCTION

Huntington's disease (HD) is a neurological disorder caused by a genetic mutation in the IT15 gene. The gene encodes for a protein called huntingtin, and the mutation results in an elongated stretch of glutamine near the NH2 terminus of the protein. It generally affecting people of middle age, progressive cell deaths in the striatum and cortex, and accompanying declines in cognitive, motor, and psychiatric functions, are characteristic of the disease[1]. Huntington's disease has a worldwide prevalence of 5 to 8 per 100,000 people, with no gender preponderance. The highest frequencies of HD are found in Europe and countries of European origin. The lowest frequencies are found in Africa, China, Japan, and Finland. Age of onset is typically between 35 and 40 years[2,3] but there is sufficient variability to support a distinction between a juvenile variant with onset before age 20 and a late onset variant principally evident after the fourth decade of life.

Numerous factors like excitotoxicity, oxidative stress, and energy impairment have been implicated the etiopathogenesis of Huntington's disease.It is generally caused by a genetic defect on chromosome[4]. The defect causes a part of DNA, called a CAG repeat, to occur many more times than it is supposed to. Normally, this section of DNA is repeated 10 to 28 times. But in persons with Huntington's disease, it is repeated 36 to 120 times, leading to the production of mutant htt protein with an abnormally long polyglutamine repeat. The mutated protein aggregates in striatal neurons as well as neurons in other regions of the neuraxis, such as the cortex, thalamus, hypothalamus, and the substantia nigra pars compacta. Mutant htt has a toxic gain of function that causes cell death via mechanisms that remain unclear .As the gene is passed down through families, the number of repeats tends to get larger. The larger the number of repeats, the greater your chance of developing symptoms at an earlier age. So, researchers are using animal models of to study the Huntington's disease (HD) disease pathogenesis, to elucidate areas of the brain involved in structural and functional decline, and to evaluate potential therapeutic interventions[5].

ANIMAL MODELS FOR HUNTINGTON'S DISEASE

Excitotoxic Models

Many animal models mimic HD symptoms or pathology .Characterization of multiple animal models is necessary for understanding the pathogenesis and the effects of potential therapies. Most animal models of HD fall into two broad categories, in general terms, excitotoxicity is dependent on the increase in the intracellular concentration of Ca^{2+} after its influx through the NMDA receptor channel. Calcium-activated enzymes such as proteases, endonucleases and phospholipases contribute to the degradation of different cell components and neuronal death. Intracellular concentration of Ca^{2+} is regulated by energy-dependent systems cytoplasmic and endoplasmic reticulum Ca^{2+}-ATPases. Massive influx of Ca^{2+} ions during excitotoxicity leads to intramitochondrial Ca^{2+} overload, altering its activity and disrupting ATP production[5]. As a consequence, ATP-dependent Ca^{2+} loading and extrusion mechanisms are impaired leading to a sustained increase in intracellular Ca^{2+}. Another key event involved in excitotoxic death is the generation of free radicals, as a result of mitochondrial dysfunction and the activation of Ca^{2+}-dependent enzymes such as xanthine oxidase and nitric oxide synthase[6]. Free radicals will damage lipidic and proteic components of the cell as well as nucleic acids, contributing to the neuronal death cascade.

Quinolinic Acid: Quinolinic acid is an endogenous metabolite derived from the kynurenine pathway of tryptophan degradation, and its implication in neuronal death associated with HD has been studied for several years. Quinolinic acid is incapable of crossing the blood-brain barrier and is therefore experimentally administered directly to the striatum[7]. Direct administration of quinolinic acid, an endogenous agonist of NMDA receptors, in the rat striatum causes the death of MSN and reproduces some of the behavioral and biochemical alterations of HD. Postmortem analysis of brains from HD patients at early stages has revealed increased levels of quinolinic acid and its precursor 3-hydroxy-kynurenine (3-HK)[8]. Also, increased activity of

the enzyme 3-hydroxyanthranilic acid oxygenase (3-HAO) has been reported in the striatum of postmortem brains[9]. This enzyme catalyzes the conversion of 3-hydroxyanthranilic acid to quinolinic acid.

Kannic acid: Kannic acid (KA) is an epileptogenic and neuroexcitotoxic agent by acting on specific kainate receptors (KARs) in the CNS. KA has been extensively used as a specific agonist for ionotrophic glutamate receptors (iGluRs). Systemic intravenous or intraperitoneal, intranasal, or local administration of KA results in a series of clinical manifestations and pathological changes in rodents, including recurrent seizures, behavioral changes of rodents, oxidative stress including the generation of ROS and reactive nitrogen species (RNS), hippocampal neuronal death and glial activation, and so forth. Upon binding to KARs (receptors), KA induces a number of cellular events, including the influx of cellular Ca2+, production of reactive oxygen species (ROS), and mitochondrial dysfunction leading to neuronal apoptosis and necrosis[10].

Energy metabolism deficit Models

There is substantial evidence that impairment of energy production may play a role in the pathogenesis of neurodegenerative diseases[11,12]. Huntington's Disease patients face pronounced weight loss, despite sustained caloric intake, which was a first indication that alterations in energetic metabolism could play a role in Huntington's Disease pathogenesis. In agreement with this hypothesis, Huntington's disease patient's exhibit alterations in cerebral glucose consume, lactate levels, and mitochondrial enzymes activity involved in glucose metabolism. Therefore, any alteration in energy metabolism represents a potential risk for loss of neuronal viability[13]. In order to reproduce metabolic alterations observed in HD at experimental levels, different animal models have been designed .Among them we can mention those of malonate and 3NP. Other models were generated starting from the observation that deficits in energy metabolism also occur in HD. To selectively target mitochondrial function various mitochondrial toxins were used, such as rotenone), 1-methyl-4-phenylpyridinium (MPP), 3-acetylpyridine and 3-nitropropionic acid (3-NP)[14].

3-Nitropropionic Acid: 3-Nitropropionic acid (3-NP) is a well-known fungus toxin causing neurotoxicity in animals and humans[15]. Systemic injections of 3-nitropropionic acid (3-NP) in rodents cause striatal neuropathy similar to that observed in clinical Huntington disease. This treatment produces a variety of neurobehavioral abnormalities and motor deficit such as bradykinesia, muscles weaknesses and rigidity[16].The brain lesions produced by the systemic administration of 3-np to laboratory animals show high specificity for the striatal tissue, although hippocampus ,thalamus and cortex are also affected[17].These observations lead to consider this protocol as a relevant model of Huntington disease and suitable model for disruption of energy metabolism ,more specifically as model of HD. The primary mechanism of action of this toxin involves the inhibition of complex II succinate dehydrogenase SDH at the mithichondrial membrane and is responsible for oxidation of succinate ato fumarate. when the enzymes is irreversibly blocked by 3 NP, decreased levels of atp and neuronal cell death process may be observed[18,19]. Thus, 3-NP toxicity leads to a depletion of ATP and energy failure, resulting in altered calcium homeostasis, excitotoxic events and neuronal death. In turn, excitotoxic cell death has been related with reactive oxygen species (ROS) formation and oxidative stress[20]. 3-NP crosses the blood-brain barrier and can be administered systemically to rats, mice, and nonhuman primates. Both the subcutaneous osmotic pump and direct subcutaneous injection are effective for the systemic administration of 3-NP to the rat, but the injection delivers a more precise dose as it is adjusted daily for the animal's weight. This feature is crucial for 3-NP dosing, as a rat's weight can decrease by as much as 20 grams per day during 3-NP administration[21]. 3-NP causes cell death by a combination of necrosis and apoptosis, both of which are seen in the HD brain.

Malonate: Malonate is a another succinate dehydrogenase that causes motor selective inhibitor of succinate dehydrogenase that couses motor impairments and neuronal pathology resembling HD after intrastriatal administration in rodents malonate does not cross blood brain barrier similar to 3 NP malonate produces age dependent striatal lessions that can be significantly attenuated NMDA receptor antagonists.Malonate induces mitochondrial potential collapse, mitochondrial swelling, cytochrome c (Cyt c) release and depletes glutathione (GSH) and nicotinamide adenine dinucleotide coenzyme (NAD(P)H)stores in brain-isolated mitochondria. Although, mitochondrial potential collapse was almost immediate after malonate addition, mitochondrial swelling was not evident before 15 min of drug presence. This latter effect was blocked by cyclosporin A (CSA), Ruthenium Red (RR), magnesium, catalase, GSH and vitamin E.4 Malonate added to SH-SY5Y cell cultures produced a marked loss of cell viability together with the release of Cyt c and depletion of GSH and NAD (P) H concentrations. All these effects were not apparent in SH-SY5Y cells over expressing Bcl-xL.When GSH concentrations were lowered with buthionine sulphoximine, cytoprotection affordedby Bcl-xL over expression was not evident anymore. Taken together, all these data suggest that malonate causes a rapid mitochondrial potential collapse and reactive oxygen species production that overwhelms mitochondrial antioxidant capacity and leads to mitochondrial swelling. Further permeability transition pore opening and the subsequent release of proapoptotic factors such as Cyt c could therefore be, at least in part, responsible for Malonate-induced toxicity[22].

Genetic Models for Huntington's disease

Although toxic models greatly contributed to the understanding of principal mechanisms underlying cell death in HD, advanced studies on the progression of the disease have been made possible after the discovery of htt gene in 1993 and recent development of transgenic models brings new alternatives for the study of possible toxic mechanisms occurring in HD from the different transgenic models available, R6/1 and R6/2 mice are among the most explored genetic models as yet[23,24,25]. The possibility to reproduce htt mutation and create more faithful paradigms of the disease allows the dissection of early events in neuronal degeneration in the different genetic mouse lines can be subdivided based on how the mhtt is incorporated into the mouse genome in: (a) transgenic mice that, besides expression of both alleles of murine wild-type htt, express also a fragment of the human htt gene containing polyglutamine mutations, like R6/2 and R6/1 mice[26,27]; (b) knock-in mice with pathogenic CAG repeats inserted into the existing CAG expansion of wild-type murine htt[28,29,30];(c) mice that express the full-length human HD gene, like mice expressing mhtt through yeast artificial chromosomes (YAC) or bacterial artificial chromosome (BAC)[31,32]; (d) mice in which mhtt can be turned on or off at a certain age,

mimicking distinct phases of the disease and allowing to dissect narrow effects of htt mutation on specific molecular targets[33,34] traducing the idea that motor symptoms and cell death might be preceded by neuronal dysfunctions in the affected brain areas. With the discovery of the htt mutation in 1993, it became possible to create animal models with a similar genetic background of the disease seen in humans with HD. The first mouse model of Huntington's disease was created in the laboratory of Gillian bates[35] since then various mouse model has been generated .These models include transgenic with truncated and full length human mutant gene), knock in and knock out models. Transgenic animal models of HD provide new ways of studying the neuropathological mechanisms underlying HD.

Knock in mouse models Huntington's disease: Knock-in mouse models are considered the most accurate for HD from a genetic standpoint. Knock-in models are useful tools to evaluate the ability of potential treatments to delay the onset of early abnormalities.It has been argued that knock-in models are more faithful in terms of genetic context and in recapitulating the late onset, slow natural progression and neuropathology of HD. Creation of these mice involves replacing a portion of the mouse htt gene with a mutant human copy that contains an expanded CAG region. Thus these mice have only two copies of the htt gene—a wild-type allele and a mutant allele, both under control of the mouse htt promoter. Initial studies in knock-in mice were disappointing because the mice did not show the overt neuropathology or symptoms seen in the transgenic mice[36]. This initial disappointment was later dispelled after closer analysis and generation of additional models. Knock-in mice are considered a precise genetic HD mouse model because they express the mutation in the murine huntingtin protein and the endogenous murine promoter controls its level of expression. Knock-in with 48, 90, and 109 CAG repeats are in Sv129 x CD1 background, 26 whereas knock-in with 71, 94, 140 are in Sv129 x C57Bl/6J background,13,19 knock-in with 80 CAG and with 150 CAG repeats are in 129/Ola x C57Bl/6J,18 and knock in with 72 and 80 CAG repeats are in Sv129 x C57Bl/6J and Sv129 x FVB/N.9,27 are the models of HD[37].

Knock out mouse models for Huntington's disease: Although knock-out mouse models are not a viable model of HD, studying these early models did increase understanding of the disease. Showing that a complete absence of the huntington gene, and consequently the huntingtin protein, was embryonic lethal revealed that Huntington's disease is not a loss-of-function disease. The ability to remove or alter with precision a single one of the thousands of genes in the body and to transmit this mutation to all subsequent progeny was a science-fiction dream only a few years ago. But today this technique is part of a routine procedure for creating animal models that can be used to study the pathophysiology and therapy of diseases in humans.To create an animal model of an autosomal recessive disease, however, both alleles of the normal gene must be inactivated. The technique of gene "knockout" was developed for this purpose. The goal of the gene-targeting (knockout) method is to replace the specific gene of interest with one that is inactive, altered, or irrelevant. To increase the probability that such replacement will occur, rather than nonspecific random integration of the DNA, both ends of the replacement gene are flanked by long DNA sequences homologous to the sequences flanking the target gene. Consequently, a knockout mouse corresponding to a particular genetic disorder may help clarify the mechanism of the disease. For example, mice with homozygous knockout of the gene for Huntington's disease die as embryos instead of undergoing the clinically predicted postnatal degeneration of their brains. This result indicates that the abnormal expansion of nucleotides in the gene of patients with Huntington's disease causes a deleterious gain, rather than a loss, of function as would be expected with an autosomal dominant disorder. In addition, a growing number of disorders due to single-gene mutations (for example, Duchenne's muscular dystrophy, cystic fibrosis, and Huntington's disease) are being discovered entirely through the chromosomal location of their defects (positional cloning), with little or no prior knowledge of the pathogenesis of the diseases[38].

Transgenic monkey model Huntington's disease: First genetically altered monkey model that replicates some symptoms observed in patients with Huntington's disease, according to a newer study. Researchers are now able to better understand this complex, devastating and incurable genetic disorder affecting the brain, could lead to major breakthroughs in the effort to develop new treatments for a range of neurological disease. Researchers have developed a rhesus macaque model of a specific human disease using transgenic technologies; a marked improvement over mouse models. Transgenic monkey model is developed by introducing altered forms of the Huntington gene into monkey eggs using a viral vector. The eggs were fertilized and the resulting embryos were introduced into surrogate mothers, resulting in five live births. The investigators are now studying the onset of the disease and its behavioural and cognitive effects, with the goal of using the monkey model to better understand disease mechanisms and to design therapies. Research team produced the HD transgenic rhesus macaques by:

- A lentivirus expressing the mutant htt gene with expanded polyglutamine repeats, which is the primary cause of HD, and
- A lentivirus expressing a green fluorescent protein (GFP) gene;
- Fertilizing the oocytes by intra cytoplasmic sperm injection (ICSI); and
- Transferring 30 embryos into eight surrogates.

This resulted in six pregnancies and five live births (two sets of twins and one singleton); all carried the mutant htt and GFP genes. Two continue to survive.

Genetic advances make it easy to identify who has inherited the disease gene. The investigators are now studying the onset of the disease and its behavioural and cognitive effects, with the goal of using the monkey model to better understand disease mechanisms and to design therapies.

Fly models of Huntington's disease: The fly is one of the best invertebrates for modeling higher organisms. Comparative genome analysis reveals that at least 50% of fly genes have similar genes in man (blast cutoff value of E<10_10)[39]. Among those human genes known to be associated with disease, 75% have a *Drosophila* ortholog[40]. The fly is also an excellent choice for modeling neurodegenerative diseases because it contains a fully functional nervous system with an architecture that separates specialized functions such as vision, olfaction, learning and memory in a manner not unlike that of mammalian nervous systems[41,42,43]. The dominant neurodegenerative diseases are particularly well suited for modeling in Drosophila because they are caused by gain of function mutations in single genes

that can readily be engineered to be expressed in flies and cause phenotypes that closely mimic the human disease. Foreign genes are expressed using a bipartite gene expression system[44,45] in which genes inserted behind the yeast upstream activator sequence (UAS) are activated by the yeast Gal4 protein. Genes fused to UAS and injected into embryos with a helper element integrate into the chromosome producing transgenic lines carrying the UAS>transgene. Unlike DNA integration events in some other systems that lead to multiple tandem insertions, the Drosophila system favors the insertion of a single transgene at a random site. Transgenic animals are then crossed to 'driver lines' that express Gal4 in a variety of tissue-specific patterns. A large collection of 'Gal4 drivers' is available[46]. For any given Gal4driver/UAS>transgene combination, it is frequently observed that using the same driver to drive different isolates of the same uas>transgene gives somewhat different phenotypes, often referred to as strong, medium and weak. It is assumed that these differences are due to position effects, where the different transgene insertion sites are expressed at higher or lower levels due to surrounding chromatin sequences, although this assumption is rarely tested temperature[47]. One widely used driver for neurodegeneration studies is elav, which expresses Gal4 in every cell of the nervous system from embryogenesis onward (Robinow S and White K.1988) [48] and another is gmr, which expresses in all cells of the eye including both neurons and surrounding supporting cells[49]. Expression of both elav and gmr is activated at the front of a morphogenetic wave that occurs in the eye disc and creates a gradient of neurons that have been exposed to toxic polyQ proteins for defined periods of time. Expression of polyQ containing proteins by elav can lead tothe degeneration of the neurons, but this is not accompanied by any overt external dysmorphology. On the other hand, expression of transgenes with the gmr driver leads to extensive degeneration in the eye and is often evident as external dysmorphology[50-53]. A caveat in the interpretation of these phenotypes is that the development of the eye depends on the stepwise specification of particular cell fates, which requires the continued contact between cells of different fates[54].Consequently, care must be exercised in interpreting neuronal cell death in settings in which the support cells are also subject to degeneration. Motor function is readily addressed by exploiting the negative geotropic behavior of flies and counting the number of time[55,56].

Yeast models for Huntington's disease: Although several transgenic animal models exist for studying the functions of Htt, none are as readily amenable to genetic analysis as yeast. Yeast models of HD have been created primarily by transgene approaches using glutamine-encoding trinucleotide expansions. To provide genetically tractable model system for the study of Htt, Krobitsch *et al.* engineered yeast cells to express an N terminal fragment of Htt with different polyQ repeat lengths. Homopolymeric tracts of CAG, the naturally occurring glutamine codon in Htt, are inherently unstable, and particularly so in yeast. To reduce this problem, the CAG and Cytosine Adenine Adenine (CAA)-mixed codon polyQ repeats were performed in their experiments according to the fact that glutamine is encoded by both CAG and CAA and that mixed-codon repeats are considerably more stable[57,58,59]. The results indicate that the extent of aggregation varies with the length of the polyQ repeat. At the 2 extremes, mostHtQ103 protein coalesced into a single large cytoplasmic aggregate, whereas HtQ25 exhibited no sign of aggregation. Further-more, the polyQ fragments of Htt exhibited minimal toxicity in yeast, whether they were present in the aggregated or soluble state [60]. In humans, Htt proteins with the longer repeat expansions not only produce disease earlier, but also expand the cell-type distribution of toxicity. Lack of toxicity for the aggregated and soluble Htt fragments in yeast is advantageous for 3 reasons. First, it provides an opportunity to study natural cellular factors that control the fate of misfolded polyQ proteins and to search for potential therapeutic agents that complication of deciphering the contributions of toxicity tothe outcome of the assay. Second, polyQ-expanded proteins may perturb the distribution of other cellular proteins by providing novel interaction surfaces[61]. The nature of these interactions is most readily and rapidly tested in an environment where the aggregation state of the polyQ protein can readily be manipulated. Finally, the greatest conundrum of the polyQ diseases may be that most of the proteins are ubiquitously expressed, yet each manifests its toxicity in unique and distinct set of neurons. Yeast screens with different polyQ proteins may provide an opportunity to identify the cell type-specific factors that contribute to the unique spectrum of toxicities and subsequently, to search for factors that ameliorate their effects[62].

An ovine transgenic model for Huntington's disease (Sheep Model): The sheep, *Ovis aries* L., is an ideal alternate mammalian model for studying HD. Sheep are relatively easy to care for as they are docile and can be kept in their normal grazing environment. Importantly, they live for more than a decade, allowing for the study of the chronic effects of a full-length huntingtin expressing transgene. Moreover, sheep are expected to be a good model for pharmacological testing because their brain has a good surgical model for testing possible cell replacement approaches, or the delivery of gene therapy agents, making feasible investigations that cannot be carried out in humans. The advantage presented by this large animal model is that *in vivo* brain-imaging techniques such as PET or MRI can be used for determining anatomo-pathological changes in relation to symptoms, thus giving valuable insight into the mechanisms underlying the progression of pathological processes. HD transgenic sheep were generated using an 11.9kb *NruI-NotI* transgene fragment containing the full-length human *HTT* cDNA ligated to 1.1kb of the human *HTT* genomic DNA immediately upstream of exon 1, containing promotor elements. The 3' end of the human cDNA transgene was flanked by a portion of the bovine growth hormone (BGH) UTR containing exon 4, intron 4 and exon 5. The introduction of an intron/exon structure into the transgene was an attempt to enhance transgene expression Given that disease severityis inversely correlated with CAG repeat length, a pure CAG repeat tract of about 69 units,which with penultimate CAACAGCAACAG codons would encode a polyglutamine tract of73 residues, was chosen and inserted into the appropriate position of the full-length human *HTT* cDNA. The ~69 CAG repeat tract is found in human HD patients and may be ofsufficient length to yield evident disease phenotypes within the ovine lifespan, as a HTT polyglutamine tract of 73 units would be associated with juvenile onset of overt disease symptoms in humans[63].

Culture cell models of HD

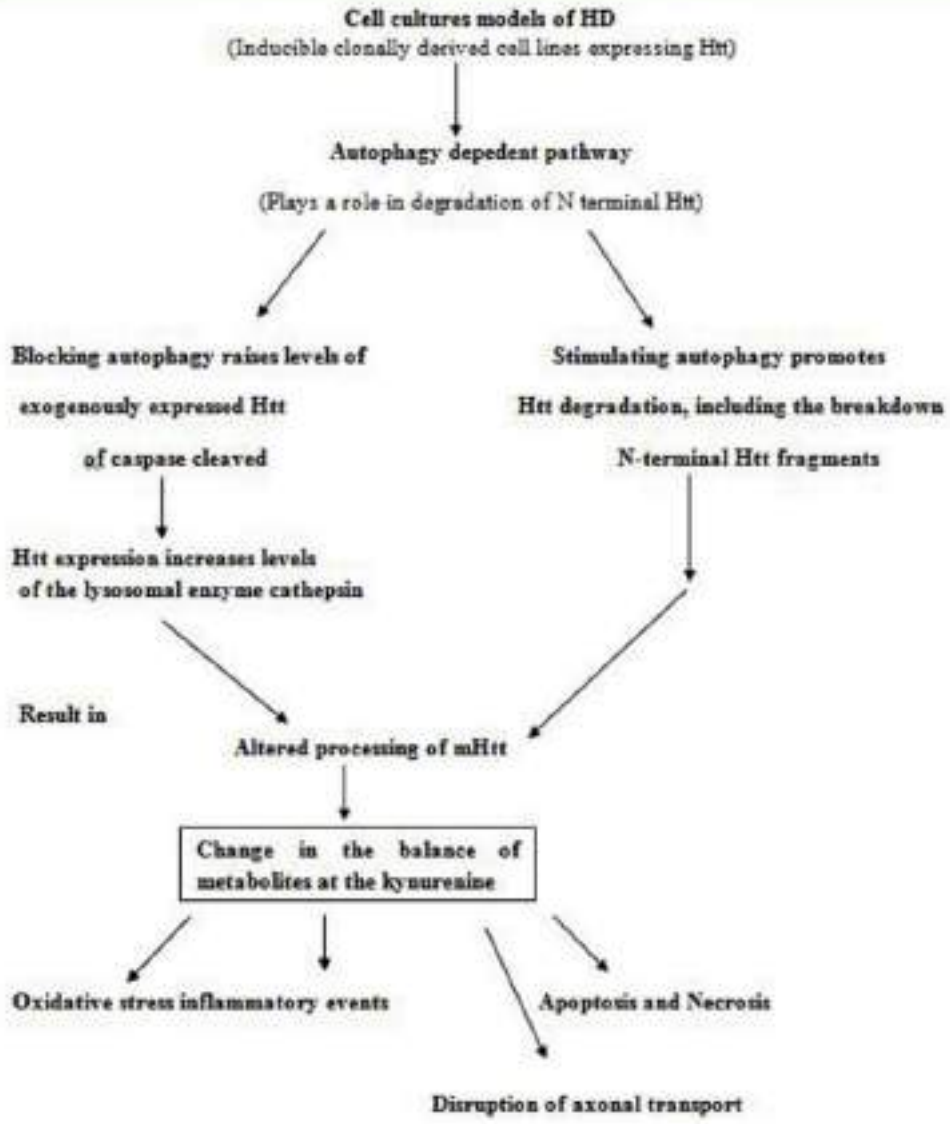
An inducible, clonally derived, cell line expressing mHtt can offer stable and controlled genetic and transcriptional backgrounds which are used in gene expression studies.

Several cell models of HD are available. One of them is a mouse neuron which has been fused with mouse teratoma cells, and the resulting hybrids have been stably transfected with various polyQ-containing peptides. These mutant polyQ lines exhibited time dependent aggregate formation, in both the nucleus and cytoplasm. Another available model is based on stably transfected, temperature-sensitive, immortalized mouse striatal neurons[64,65]. Stable, inducible PC12 models of HD expressing wild type and mutant Htt, either in the context of an exon 1 fragment or the full-length protein, have been employed. These models exhibit normal morphology and growth patterns and these cell lines expressed similar levels of endogenous and exogenous mHtt, analogous to the heterozygous condition. ST14A cells, derived from rat embryonic striatum, were also used to generate inducible cell lines expressing the N-terminal fragment of Htt. The advantage of these models is that we can study events without the complications of cell death, because cell death in the mutant, mitotic lines is very low and is no higher than that of the wild type lines[66,67]. Cell models to elucidate the molecular pathogenesis of HD have been used. Many hypotheses have been produced based on cell models studies *in vitro*. Some investigators have explored the role of autophagy in Htt processing in 3 cell lines: clonal striatal cells, PC12 cells and rodent embryonic cells lacking cathepsin D. Autophagy plays a critical role in the degradation of N-terminal Htt. Blocking autophagy raises levels of exogenously expressed Htt, reduces cell viability and increases the number of cells bearing mHtt aggregates. Stimulating autophagy promotes Htt degradation, including the breakdown of caspase cleaved N-terminal Htt fragments. They also found that Htt expression increases levels of the lysosomal enzyme cathepsin D by an autophagy-dependent pathway. These results indicate that altered processing of mHtt by autophagy and cathepsin D may contribute to HD pathogenesis[68]. The mammalian target of rapamycin is a kinase which can inhibit autophagy in cells from yeast to humans. Rapamycin is a specific inhibitor of mammalian target of rapamycin (mTOR) which can stimulate autophagy. Rapamycin is lipophilic and demonstates good blood-brain barrier penetration. These results indicate that rapamycin is a good chemical candidate for use in treating HD. The ability of rapamycin to inhibit mTOR activity may be impaired after prolonged Htt expression and thus increased aggregate formation. Therefore, early treatment with rapamycin may attenuate HD[69].

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