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Review On Ubiquitylating and Deubiquitylating Enzymes in Drug Discovery

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ABSTRACT
This article reviews the emerging opportunities of ubiquitylating and deubiquitylating enzymes on drug discovery, mainly focuses on deubiquitylating enzymes. Drug discovery is the process through which potential new medicines are identified. The process of **drug** discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy.Once therapeutic area has been identified the next stage is to identify a suitable drug target .An understanding of which bio macromolecule is involved in a particular disease is clearly important. This allows to identify the agonist and antagonist should be designed to a particular receptor, or whether inhibitors should be designed for a particular enzyme. Ubiquitylation and related processes control various aspects of human cell biology and physiology.Defects in such processes can leads to several diseases.DUBs deregulation contributes to various genetic disorders including cancer.Components of ubiquitylation machinery can be taken as drug

KEYWORDS: Drug discovery, ubiquitin, ubiquitin proteasome system, DUBs, Deubiquitylation

I. INTRODUCTION

The word ‘drug molecules’ has been appropriately and judiciously employed in line with the classical concept, that it’s a chemical entity which essentially possesses a significant and pronounced pharmacological activity, emphatically on the mammalian organism. In fact, it is the dedicated and collaborated efforts of expert researchers from various scientific fields, where, a new molecule is isolated, characterized and subsequently subjected to a rigorous preclinical and successful clinical studies, and ultimately led to a therapeutically effective, potent ‘drug’ molecule with minimal side effects.

Drug discovery is the process through which potential new medicines are identified. The process of **drug** discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Once a compound has shown its value in these tests, it will begin the process of **drug** development prior to clinical trials. A huge investment has to be made towards the research and development of a new drug. As result research projects tends to focus on diseases that are important in the developed world .A great deal of research is carried out on ailments such as depression, ulcer, cancer and cardio vascular disorders .Less is carried out on tropical diseases.

Once therapeutic area has been identified the next stage is to identify a suitable drug target .An understanding of which bio macromolecule is involved in a particular disease is clearly important. This allows to identify the agonist and antagonist should be designed to a particular receptor, or whether inhibitors should be designed for a particular enzyme. For example tricyclic anti-depressant is known to inhibit the uptake of nor adrenaline from nerve synapses by inhibiting neither carrier protein for nor adrenaline. However these drugs also inhibit uptake of serotonin and the possibility arose that inhibiting serotonin uptake might also be beneficial.

STEPS IN DRUG DISCOVERY

1. Choosing a disease

Pharmaceutical companies must make a profit to exist .Pharmaceutical companies will, therefore, avoid products with too small a market (i.e. a disease which only affects a small subset of the population) Pharmaceutical companies will also avoid products that would be consumed by individuals of lower economic status (i.e. a disease which only affects third world countries) . Most research is carried out on diseases which afflict “first world” countries: (e.g. cancer, cardiovascular diseases, depression, diabetes, flu, migraine, obesity)

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2. Choosing a drug target

Once a therapeutic area has been identified the next stage is to identified a suitable drug target .An understanding of which biomolecules are involved in a particular disease state important. For example a search for selective serotonin uptake inhibitor leads to discovery of fluoxetine.

3. Identifying bioassay

In vitro testing

• Has advantages in terms of speed and requires relatively small amounts of compound. • Speed may be increased to the point where it is possible to analyze several hundred compounds in a single day (high throughput screening). • Results may not translate to living animals

In vivo tests

• More expensive • May cause suffering to animals • Results may be clouded by interference with other biological systems.

4. Preclinical Studies in animals

• Pharmacodynamics: To explore actions relevant to the proposed therapeutic use • Pharmacokinetics: how the drug is distributed in and disposed of by the body • Toxicology: whether and how drug causes injury (in vitro tests and intact animals) -- single-dose studies - acute toxicity -- repeated dose studies – sub-acute chronic or long term toxicity --- Done in 2 species – rodent and non-rodent --- Clearance from Institutional Animal Ethic Committee required.

5. Clinical testing (trials)

• Phase I – Human Pharmacology (Healthy volunteers – 20-50 subjects) • Phase II - Therapeutic Exploration (patients – 50- 400) • Phase III – Therapeutic Confirmation (large scale multi-centre; 250-1000) • Phase IV - Therapeutic Use (post- registration monitoring) {Phase 0, Microdosing} .

DISCOVERY OF DRUG TARGET

Target discovery, which involves the identification and early validation of disease-modifying targets, is an essential first step in the drug discovery pipeline. A **drug target** is a molecule in the body, usually a protein, that is intrinsically associated with a particular disease process and that could be addressed by a **drug** to produce a desired therapeutic effect. If a drug has a biological effect, there must be a drug target for the drug. On the basis of existing knowledge, we were able to determine that all current drugs with a known mode-of-action act through 324 distinct molecular **drug targets**. Of these, 266 are human-genome-derived proteins, and the remainders are bacterial, viral, fungal or other pathogenic organism **targets.** Modern drug discovery often involves screening small molecules for their ability to bind to a preselected protein target. Target-oriented syntheses of these small molecules, individually or as collections (focused libraries), can be planned effectively with retrosynthetic analysis. Drug discovery can also involve screening small molecules for their ability to modulate a biological pathway in cells or organisms, without regard for any particular protein target. This process is likely to benefit in the future from an evolving forward analysis of synthetic pathways, used in diversity-oriented synthesis that leads to structurally complex and diverse small molecules. One goal of diversity-oriented syntheses is to synthesize efficiently a collection of small molecules capable of perturbing any disease-related biological pathway, leading eventually to the identification of therapeutic protein targets capable of being modulated by small molecules.

In the past the discovery of a drug target depends on finding the drug first. Many early drug such as morphine, are natural products derived from plants and just happened to interact with a molecular target in the human body. As this involved coincidence more than design, the detection drug target was very much a hit and miss affair.Later the body's own chemical messenger started to be discovered and pointed the finger at further target.Despite this relatively few of the body's messengers were identified, either because they were present in such small quantities or because they were too short lived to be isolated.

Indeed many chemical messengers still remains undiscovered. This in turn means that body's potential drug targets remain hidden. The various genome projects which have mapped the DNA of humans and other life forms, along with the newer field of proteomics are revealing an ever increasing number of new proteins which are potential drug targets for the future.

NUCLEIC ACID AS DRUG TARGET

Nucleic Acids

Nucleic acids are another class of important drug targets. They are of particular significance in the medicinal chemistry of certain anticancer drugs and gene silencing therapeutics. DNA and RNA are nucleic acids. Just like proteins, nucleic acids are polymeric macromolecules. The

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monomeric units of nucleic acids are referred to as **nucleotides**.

Nucleotides are made up of at least one phosphate group, a pentose (5-carbon sugar), and a heterocyclic nitrogenous base (referred to as **nucleobases**). For DNA, the pentose is deoxyribose whereas for RNA, the pentose is ribose. The nucleobases are adenine, thymine, guanine, cytosine, and uracil (RNA). The nucleobases are further divided into **purines** and **pyrimidine**. When the nucleobase is covalently bonded to the pentose alone, it is referred to as a **nucleoside**. Structures and the numbering system are shown in the image below. To summarizes:

- **Nucleobase:** Nucleobase alone
- **Nucleoside:** Nucleobase + Pentose
- **Nucleotide:** Nucleobase + Pentose + Phosphate(s)
- **Nucleic Acid:** Polymerization of nucleotides. Nucleotides are linked through 3'-5' phosphodiester linkages

DNA essentially carries the genetic information of an organism. Interfering with DNA can be detrimental to a cell. The drugs that interact with DNA can be grouped into:

- Groove Binders
- Intercalators
- Alkylating Agents
- Chain Terminators
- Chain Cutters

ENZYME AS DRUG TARGET

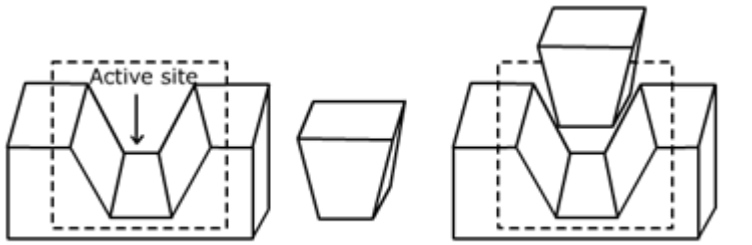
Enzymes are the proteins in the drug design that act as drug targets for the diseases in the process of drug discovery and development. There are number of drug targets involved in the designing of the drug.

Drug target as a nucleic acid or a protein (e.g. an enzyme, a receptor) whose activity can be modified by a drug. The drug can be a small-molecular-weight chemical compound or a biological, such as an antibody or a recombinant protein. The drug target should have been shown to be effective/mechanistically involved in the disease by relevant in vitro or in vivo models.

1. How do enzymes catalyst the reaction:-

i. The first function of an enzyme is to hold the substrate molecule for a chemical reaction. The active site of enzymes holds the substrate molecule in suitable position so that it can be attacked by reagent effectively.

ii. The second function of enzymes is to provide functional groups which will attack the substrate to carry out the chemical reaction. The amino acid present on active site of enzyme provides free amino group to attack the substrate and bring the chemical reaction.



2. Drug enzyme interaction:-

i. Drugs which inhibit any of the two activities of the enzyme are called the inhibitors they block the binding site thus preventing the binding of the substrate to active site.

ii. Drugs which compete with the natural substrate for their attachment on the active sites of enzymes are called competitive Inhibitors.

iii. Some drugs do not binds to the active sites but binds to a different site of the enzyme called allosteric site

iv. In the bond formed between an enzyme and the drug (inhibitor) is a strong covalent bond. Then enzyme is blocked permanently.

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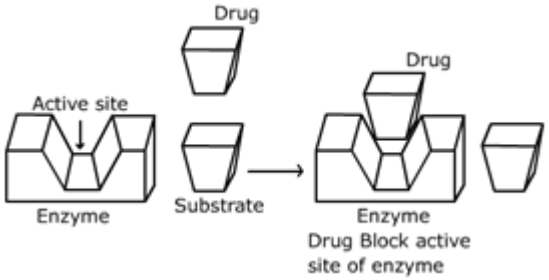

v. Drug and substrate competing for active site.

Receptors:-
They are the proteins which are crucial to the communication system in the body. They present in cell membranes small parts of their possess the active site out of surface of the cell membrane.

Chemical messenger:-
In the animal body, the message between two neurons and muscles in communicated through certain chemical substances called the chemical messengers. To accommodate these chemical messengers the shape of receptor protein changes and messenger give the message to cell without entering into it.

Type of chemical messengers:-
a. Hormones:-
They are a group of biomolecules which are produced the ductless (endocrine) glands they enters the blood stream and travel in the body to activate all receptors to attend their message.
b. Neurotransmitters:-
Nerve transfer message through neurotransmitters. They are small molecules such as acetylcholine, dopamine and serotonin.
c. Why drug causes side effect:-
Side effect of drugs is caused when a drug binds to more than one site of receptor. Example some anti depressant drugs binds to serotonin receptor.

Ubiquitin

Ubiquitin is a small protein that is found in almost all cellular tissues in humans and other eukaryotic organisms, which helps to regulate the processes of other proteins in the body. **Ubiquitin** affects cellular process by regulating **the** degradation of proteins (via **the** proteasome and lysosome), coordinating **the** cellular localization of proteins, activating and inactivating proteins, and modulating protein-protein interactions. Protein degradation through the **ubiquitin** proteasome system (UPS) is the major regulator of programmed protein destruction in human **cells** and plays an outsized **role** in controlling **cell cycle** progression . However, the **ubiquitin** system is also tightly linked to G1/S regulation in normal and cancer **cells**. **Ubiquitination** is a process through which **ubiquitin** molecules are attached to protein substrates for protein degradation. It is one of the most **important** posttranslational modifications (PTMs) regulating the stability and functional activity of proteins. **Ubiquitin** is first **activated** by **ubiquitin-activating** enzyme 1 (UBE1), followed by conjugation to **ubiquitin**-conjugating enzyme E2, and ligation to lysine residues of specific proteins by **ubiquitin** protein ligase E3. **Proteasomes** are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins. Proteins are tagged for degradation with a small protein called **ubiquitin**. The tagging reaction is catalyzed by enzymes called **ubiquitin** ligases.

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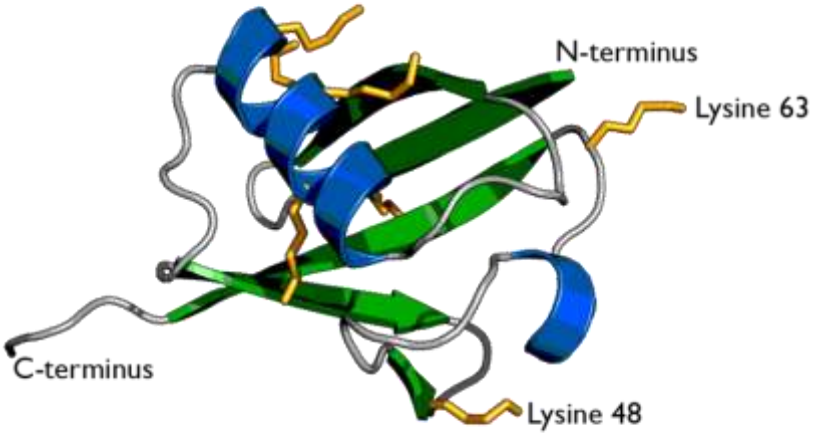

UBIQUITIN PROTEASOME SYSTEM

The ubiquitin-proteasome system (UPS) is a crucial protein degradation system in eukaryotes. Herein, we will review advances in the understanding of the role of several proteins of the UPS in Alzheimer's disease (AD) and functional recovery after spinal cord injury (SCI). The UPS consists of many factors that include E3 ubiquitin ligases, ubiquitin hydrolases, ubiquitin and ubiquitin-like molecules, and the proteasome itself. An extensive body of work links UPS dysfunction with AD pathogenesis and progression. More recently, the UPS has been shown to have vital roles in recovery of function after SCI. The ubiquitin hydrolase (Uch-L1) has been proposed to increase cellular levels of mono-ubiquitin and hence to increase rates of protein turnover by the UPS. A low Uch-L1 level has been linked with Aβ accumulation in AD and reduced neuroregeneration after SCI. One likely mechanism for these beneficial effects of Uch-L1 is reduced turnover of the PKA regulatory subunit and consequently, reduced signaling via CREB. The neuron-specific F-box protein Fbx2 ubiquitinates β-secretase thus targeting it for proteasomal degradation and reducing generation of Aβ. Both Uch-L1 and Fbx2 improve synaptic plasticity and cognitive function in mouse AD models. The role of Fbx2 after SCI has not been examined, but abolishing ß-secretase reduces neuronal recovery after SCI, associated with reduced myelination. UBB+1, which arises through a frame-shift mutation in the ubiquitin gene that adds 19 amino acids to the C-terminus of ubiquitin, inhibits proteasomal function and is associated with increased neurofibrillary tangles in patients with AD, Pick's disease and Down's syndrome. These advances in understanding of the roles of the UPS in AD and SCI raise new questions but, also, identify attractive and exciting targets for potential, future therapeutic interventions.

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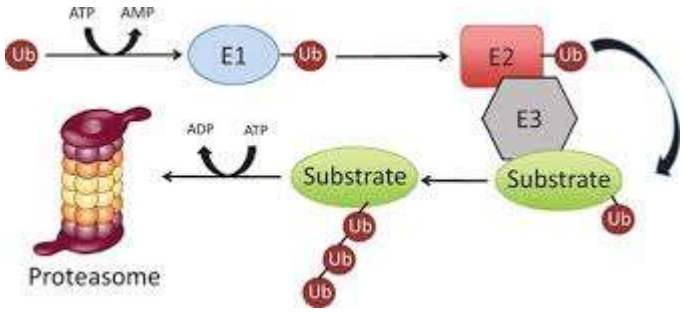
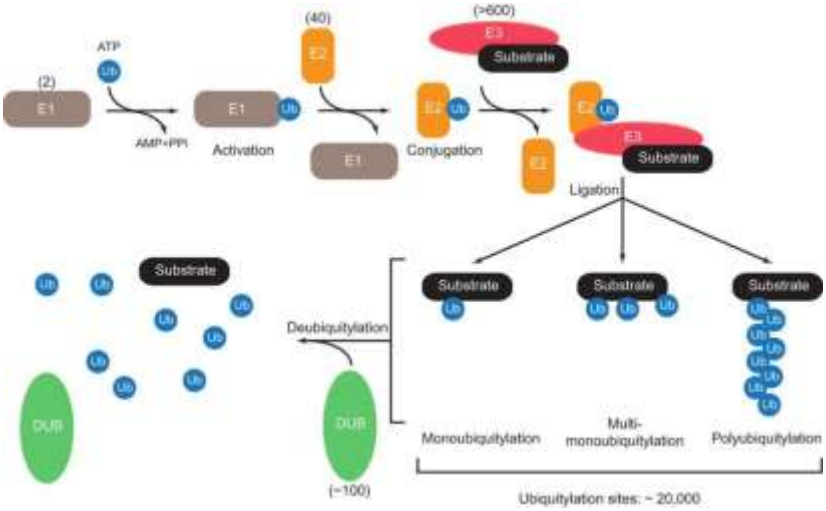


DEUBIQUITIN

- **Deubiquitylating enzymes** are proteases responsible for removing ubiquitin and ubiquitin chains from proteins.
- **Deubiquitylating enzymes** are either cysteine proteases or metalloproteases. As ubiquitylation of proteins can regulate their delivery to degradative proteasomes or to subcompartments of the cells, and regulate protein–protein interactions, deubiquitylating enzymes have consequences on substrate stability, localization and function.
- The deubiquitylating enzyme (DUB) family contains ~100 proteins that remove the post-translational modification ubiquitin from a variety of substrates.
- DUBs have key roles in various areas of cell biology of high relevance to pathologies such as autoimmune disorders, chronic inflammation, oncology and neurodegeneration.
- DUBs are attractive targets for small-molecule drug discovery, as they contain a well-defined active site, and the majority of them have a catalytic cysteine.
- Oxidative hydrolysis of the active-site cysteine is a challenge for DUB inhibitor screening, as reducing agents are often required to maintain DUB activity but frequently result in high false-positive rates if used at high concentrations.
- Many of the reported DUB inhibitors have been shown to be rather non-selective in biochemical selectivity profiling assays.
- Recent advances in screening substrates and technologies, as well as activity-based probes for monitoring target engagement, have facilitated progress in DUB drug discovery.
- Increased understanding of DUB biology and emerging examples of potent and selective DUB inhibitors suggest that clinical development of DUB inhibitors is on the horizon.
- Protein deubiquitination is becoming increasingly instrumental in understanding the complexities of the Up system.



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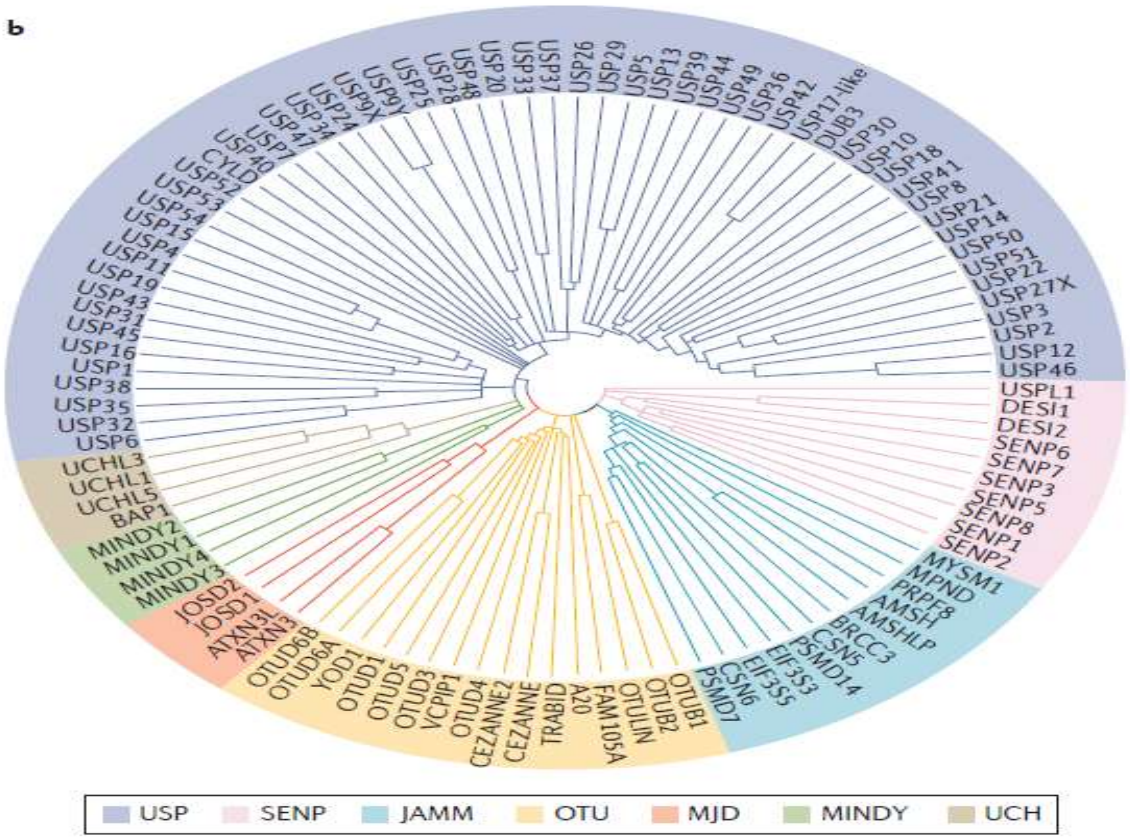
APPLICATIONS OF DUBs

DUBs in oncology

- BAP1-Tumour suppressor activity.
- USP22-Control epigenetic changes that may lead to tumour development.
- USP7,USP28-Regulate levels of tumour suppressor proteins.
- USP16-Has stem cell renewal activity.
- USP1-Repair DNA damage.USP11-complex with BRCA2 thus helps to treat breast cancer.
- USP1-Removes monoubiquitin from proliferating cell nuclear antigen ,a DNA replication component that also functions in DNA repair.

- **DUBs in DNA repair**
- USP1-Carry out DNA crosslink repairBortezomib - proteasome inhibitor- used to treat cancer.
- UCHL5 -anticancer activity - less side effects.
- **Proteasome in DUBs**
- PSMD14 -inhibition cause cancer cell sensitization to DNA damaging agent.
- USP14 -ubiquitin recycling.
- UCHL5-prevent protein degradation

TYPES OF DUBs



II. CONCLUSION

Ubiquitylation and related processes control various aspects of human cell biology and physiology.

Defects in such processes can leads to several diseases.

DUBs deregulation contributes to various genetic disorders including cancer.

Components of ubiquitylation machinery can be taken as drug

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