LESSON PLAN USING THE DEGRADATOR GAME

- high schools (age 15+)

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The goal of the DEGRADATOR game is to make students aware of the role of protein degradation in maintaining homeostasis of the intracellular environment. During the game, students take on the role of ubiquitin ligase - a protein responsible for attaching ubiquitin to proteins destined for destruction in the proteasome. Ubiquitin-tagged proteins gain affinity for the proteasome, which breaks peptide bonds between amino acids. This destroys the primary structure of the ubiquitinated protein. The product of this reaction are single amino acids or short amino acid chains, which can be reused during translation, reconstituting the molecule of the degraded protein, or forming a protein molecule of a completely different one. An additional benefit of the game is to make students aware of the probabilistic nature of enzymatic reactions and to observe the role of complexes of several enzymes in driving enzyme-substrate specificity.

Classroom preparation

The classroom should be equipped with a multimedia projector and a computer to display the presentation and game. Students should have at their disposal a computer, laptop, cell phone or other mobile device with access to the Internet. The handout is expected to last 90 minutes of class time, but due to differences in curricula at different educational institutions, it is possible to conduct the lesson in 45 minutes. The lesson is also adapted for students with special needs who cannot participate in the game. Each stage of the game has been described, and materials can be found in the downloadable files from the "For educators" section of the game's website (link on page 2).

Sample lesson scenario

1. Substantive introduction (PowerPoint presentation available for download from the "For educators" section of the game's website)
2. Solution of levels 1 and 2
3. Discuss the issues of the levels presented (what students observed during the game)
4. Level 3 quiz solution
5. Discussion of the quiz
6. Solving levels 4 and 5
7. Discuss the issues of the levels presented (what students observed during the game)
8. Level 6 quiz solution
9. Discussion of the quiz
10. Substantive introduction to levels 7 and 9
11. Discuss the issues of the levels presented (what students observed during the game)
12. Level 10 quiz solution
13. Discussion of the quiz

The DEGRADATOR game consists of 10 levels, in each of which the participant faces a with a different task that deepens his understanding of how the ubiquitin-proteasome system (UPS) works. We propose a scenario in which students first go through one level of the game independently, and then it is discussed together. A very important part of the training is the students' independent work of verbally describing what is presented at each stage. Passing successive levels before discussing them simulates the performance of experiments by scientists, who first observe a phenomenon and only then describe it in a logical way.

Before starting the activity, the teacher should familiarize themself with the educational materials for the DEGRADATOR game available in the "For educators" section at the following link: https://degradator-game.com, and in particular with the "Great Encyclopedia of Protein Degradation." The materials in the "Great

Encyclopedia of Protein Degradation" are also recommended for students, but it is not necessary to read them before participating in the class.

❖ **FIRST LEVEL** - getting acquainted with the rules of the game DEGRADATOR
Player moves with the cursor/touch (depending on whether playing on a computer or mobile device) the enzyme - E3 ubiquitin ligase. The goal is to create a four-component complex of ubiquitin - E2 enzyme - E3 enzyme - substrate. The four ubiquitin residues attached to the protein molecule are sufficient signal for its degradation by the proteasome. Player during the first level must correctly degrade four proteins in 3 minutes.

After the students have passed the first level of the game, the following issues should be addressed:

Internal environment of the cell
Very briefly remind students of the structure of the cell and the composition of the cytoplasm. Particularly important information is that the cytosol is a dense aqueous solution containing many different proteins. In this environment, one of the most important mechanisms that allow enzymatic reactions to occur is the diffusion of proteins and their substrates. It is important to emphasize that the encounter of an enzyme with a substrate is a completely random event, and to correct the frequent incorrect impression of students that substrates deliberately and directedly move toward enzymes - like iron objects attracted by a magnet. Increasing the chance of a given reaction occurring can only be done by increasing the concentration of the substrate, enzyme or raising the temperature (i.e., accelerating diffusion).

Next, make sure that students remember the mechanism of protein formation by translation. When there is a need to produce more of a substance in the intracellular environment, mechanisms are triggered that lead to the expression of genes encoding enzymes that produce this compound. This leads to an increase in the number of molecules of this enzyme in the cytosol, which translates directly into the formation of more of the desired product.

The vital needs of the cell and the organism it builds are constantly changing, so that after some time previously produced proteins may no longer be needed. This is one of the situations when such proteins can be ubiquitinated and destined for degradation in proteasomes. The amino acids thus recovered will be reused during another translation reaction to build a new protein molecule.

Nature of enzymes and enzyme complexes

Enzymes catalyze chemical reactions due to their characteristic structure. Students should be sensitized here that in the natural world, physical structure is the sole determinant of function - this is true both at the level of genes and proteins, and at the level of whole tissues and organisms. Enzyme proteins adapt to their substrates by means of the shape of their surface and the chemical nature of the amino acids that make up their active center.

Mechanisms of protein degradation in the cell

Special attention should be paid to the fact that the processes of protein synthesis and degradation are in constant balance with each other, and their disruption leads to the development of diseases associated with excessive destruction, or accumulation of proteins. The DEGRADATOR game focuses on the destruction of proteins through the UPS system, but this is not the only mechanism that allows cells to remove proteins. Mechanisms such as autophagy and the export of proteins outside the cell via extracellular vesicles play an important role. Information regarding these mechanisms is presented in the "Great Encyclopedia of Protein Degradation."

Note the differences between the various protein destruction pathways.

Autophagy - the process of packing unneeded proteins or whole cell organelles through cytoplasmic vesicles (autophagosomes), which are transported to lysosomes (organelles where intracellular digestion occurs). This mode of destruction occurs especially in situations of cell starvation, when high turnover of proteins and organelles provides the ingredients for building proteins necessary for cell survival. However, prolonged and intense autophagy can activate pathways that lead to cell death (apoptosis). The increased interest in the phenomenon of autophagy intensified when its important role in tumorigenesis was proven. Again, the autophagy phenomenon can be both beneficial and detrimental to our health. On the one hand, autophagy enables the "selection" of damaged cells and conditions their survival under harsh conditions, but on the other hand, it can enable the survival of cancer cells, which hinders healing.

Extracellular vesicles - unnecessary proteins or organelles can also be packaged into so-called extracellular vesicles (EVs). These are vesicles secreted into the extracellular space with a size not exceeding 1,000 nm. They are divided into small EVs - up to 200 nm in diameter, called exosomes, and large EVs with a diameter of more than 200 nm. Transport by EVs is crucial because it "ejects" unwanted products outside the cell, from where they can be "eaten" by immune cells, or be absorbed by other cells, altering their function. Thanks to the latter, EVs also play a significant role in intercellular communication.

UPS system - is the axis of the game. It is a highly efficient, selective and rapid system of protein destruction. It plays a key function in the regulation of cell division, apoptosis, tumorigenesis and inflammatory reactions, being responsible for the degradation of 80-90% of proteins in the cell. For discovering the mechanisms of protein degradation by the UPS system, Aaron Ciechanover, Avram Hershko and Irwin Rose received the Nobel Prize in 2004 (learn more at: https://www.nobelprize.org/prizes/chemistry/2004/popular-information).

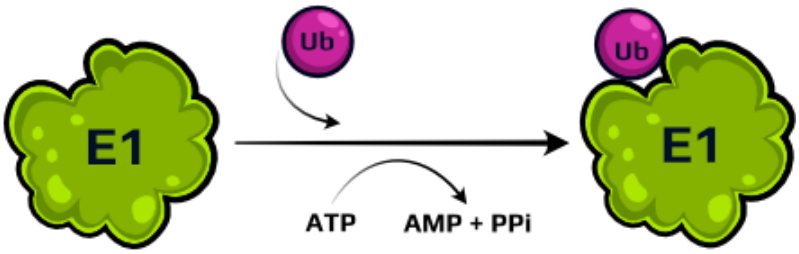
Note that the mere appearance of ubiquitin on a protein does not necessarily immediately direct the protein to degradation. A single ubiquitination rarely leads to such an effect, and the proteasome usually recognizes ubiquitin chains. Ubiquitination may also have other regulatory functions, such as affecting the localization of proteins inside the cell.

❖ **SECOND LEVEL** - protein degradation in the cell is ATP-dependent

In the second stage, player has to reassemble the enzyme complex by directing the E3 ligase, leading to the degradation of four proteins within 3 minutes. However, at this stage, they must keep in mind that ubiquitin on the E2 enzyme is indirectly regenerated as a result of energy from ATP hydrolysis. Therefore, player's catching of an ATP molecule enables the regeneration of ubiquitin on the nearest E2 enzyme. Another complication in this level is the appearance of an additional substrate that is not recognized by our E3 ligase.

Substrate specificity of enzyme reactions

Explain to students how enzymes recognize the substrates of their reactions. Point out that this is a spatial interaction, which involves matching the reaction substrate to the enzyme's active site (the substrate's binding site). This interaction changes the spatial structure of the enzyme's active site, which "stimulates" the enzyme to act. This is the so-called glove or induced matching theory, which has supplanted the once popular key-and-lock theory, in which the changes in the shape of the enzyme under the influence of interaction with the substrate have been unjustly overlooked.



Selective protein degradation requires energy from the breakdown of ATP. It is necessary for the activation of the E1 enzyme, which then transfers ubiquitin to the E2 enzyme, which, being in complex with E3 ligase, ultimately transfers it to the substrate being degraded.

ATP - the universal energy carrier in the cell

It should be noted that protein degradation processes in the cell require energy inputs from the breakdown of ATP. ATP is a universal energy carrier in the cell. To better understand this concept, we can compare it to fuel (ATP), which is necessary for the engine (enzyme) that moves the car (chemical reaction). Students should be especially sensitized to this, because the processes of protein destruction during digestion in the digestive tract do not require the supply of energy. Hence, protein degradation inside the cell is of a different nature than just "breaking" proteins into amino acids. The main difference is the selectivity of intracellular degradation and the non-selectivity of digestion.

❖ **THIRD LEVEL** - short summary QUIZ

During the quiz, player consolidates the knowledge gained by performing the tasks from previous levels.

❖ **FOURTH LEVEL** - presentation of a modern PROTAC-type drug that enables selective degradation

Player has to degrade four proteins in 3 minutes that are not normally recognized by the game's protagonist. However, by grabbing the PROTAC molecule, our E3 ligase will be able to bind new substrates.

PROTAC-type compound with apparent two-membered structure - the fragment on the left binds to E3 ligase, and the fragment on the right with a selected substrate for selective degradation.

Enzyme reactions can be controlled by activators and inhibitors

It should be noted that enzymes as catalysts of biological reactions can undergo both activation and inhibition. Activators are molecules that increase the activity of a given enzyme by increasing its affinity for a given substrate. Inhibitors, on the other hand, decrease the activity of a given enzyme by decreasing the affinity for a given substrate.

Molecular glues vs PROTAC

The above considerations are necessary for us to understand the difference between molecular glue and chimeric molecules targeting proteolysis (PROteolytic TArgeting Chimeras; PROTAC). Molecular glues are activators of the E3 ligase complex that increase the affinity of the E3 ligase for a given substrate. As a result, proteins targeted for degradation are more efficiently ubiquitinated and targeted to proteasomes. Thus, the use of **molecular glues does not change the reaction substrate, but the efficiency of the reaction.** PROTAC molecules, on the other hand, act as adapters so that we can plug a device that has a European plug into an American electrical outlet. Since, as a rule, the E3 ligase is substrate specific (it binds to specific proteins) then, in order to force it to ubiquitinate another protein, we must use an "adapter." **Therefore, PROTAC changes the reaction substrate** and we can use it when we want to get rid of a specific protein from the cell. One example is cancer, in which there is excessive production of proteins promoting their growth and invasiveness. The use of PROTAC compounds potentially enables the destruction of these proteins and slows the growth of the tumor, making it easier and more effective to cure.

❖ **FIFTH LEVEL** - PROTAC compounds enable degradation of a wide variety of substrates

Player has a variety of PROTAC compounds to ubiquitinate different substrates with the same E3 ligase.

The use of different PROTAC compounds is of great importance in the design of future anticancer therapies. With PROTACs, a specific E3 ligase (one that matches one part of the compound) gains the ability to ubiquitinate substrates other than normal. This potentially makes it possible to control the degradation of specific proteins in a patient's cells, often ones that traditional small-molecule drugs are unable to interact with.

❖ **SIXTH LEVEL** - deubiquitinating enzymes cut off ubiquitin residues, inhibiting protein degradation

Player again has to degrade four proteins in 3 minutes. However, starting from this level, they will be hindered by deubiquitinating enzymes that cut off the attached ubiquitin residues, which reduces the chance of their degradation in the proteasome.

Deubiquitinating enzymes

DeUBiquitinating enzymes (DUBs) are proteases, or enzymes that cut peptide bonds present in proteins. DUBs cut ubiquitin residues from proteins, preventing their degradation in the proteasome. Like many regulatory mechanisms in biology, they work in equilibrium with E3 ligases, which are designed to attach ubiquitin residues to proteins. In DEGRADATOR, DUBs are our adversaries that interfere with the proteasome's ability to direct proteins for degradation. Excess DUBs activity can cause disease. In some types of cancer, they can inhibit the ubiquitination of molecules signaling that promotes the growth of cancer cells, which drives tumor growth. Nonetheless, they have very important functions during cell division, thus playing an indispensable role in tissue regeneration and body growth, for example. Deubiquitinating enzymes also play an extremely important role in the course of

infections with certain viruses. They inhibit the uncontrolled ubiquitination of cellular proteins that is driven by viruses, so that the virus cannot replicate efficiently in host

cells and attack other cells. We strongly encourage you to familiarize students with a comic about DUBs that is posted on the game's website (link on the p. 2) in the “For educators” section.

❖ **SEVENTH LEVEL** - a short summary QUIZ

During the quiz, player consolidates the knowledge gained by performing the tasks from previous levels.

❖ **EIGHTH LEVEL** – inhibitors of deubiquitinating enzymes

Player again has to degrade four proteins in 3 minutes. In this level, deubiquitinating enzymes will again be interfered with, but this time a deubiquitinating enzyme inhibitor is added to the player's arsenal. When player hovers over the inhibitor, the nearest deubiquitinating enzyme is frozen.

Enzyme inhibitors

1. Before discussing this issue, students should be reminded of the basics of enzymes and their inhibitors.
2. **An inhibitor** is a molecule that inactivates a catalyst (enzyme) thereby reducing the rate at which a given reaction occurs. There are two basic mechanisms of inhibition: **competitive (substrate)** and **non-competitive**.
3. **Competitive**, or substrate, **inhibition** involves the inhibition of an enzyme by the binding of the inhibitor to the enzyme's active center. Consequently, its action can be reversed by increasing the concentration of the substrate. A different mechanism is characterized by **noncompetitive inhibition**, which binds to the enzyme at a site other than the active center (at the so-called center of the allosteric), which prevents substrate binding and results in enzyme inactivation. In the game, we do not specify which type of inhibitor we

are dealing with, because in the case of DUBs there are inhibitors that bind both reversibly and irreversibly.

❖ **NINTH LEVEL** - auto-ubiquitination of E3 ligase results in its degradation by the proteasome

Player is again faced with the task of ubiquitination of four proteins within 3 minutes, however, our hero becomes vulnerable to ubiquitination itself. If our E3 ligase remains in complex with the E2 enzyme (on which the ubiquitin is) for a long time and does not bind its substrate, the ubiquitin molecule will be transferred to it. After ubiquitination four times, our hero will be targeted for degradation and the game will end.

E3 ligases can be degraded in two ways: through a self-catalyzed process (**auto-ubiquitination**) or via modification catalyzed by other ligases. In the context of this mechanism, it is also worth noting the non-proteolytic functions of auto-ubiquitination. An example of such a function is the activation of E3 ligases, which highlights the complexity and multifunctionality of the ubiquitination process in the regulation of protein function.

❖ **TENTH LEVEL** - DEGRADATOR game summary QUIZ

The quiz summarizes the knowledge gained from the game and from the "Great Encyclopedia of Protein Degradation"

Learning Outcomes

Student:

1. defines the function and structure of proteins in the cell;
2. determines how chemical reactions occur in the cytoplasm and understands the role of the composition of the cytoplasm for its course;
3. explains the concepts of metabolic pathway (protein turnover) with examples;
4. compares the nature of anabolic and catabolic processes and demonstrates that they are related;

5. compares selective intracellular protein degradation from extracellular digestion;
6. defines the role and essence of protein turnover in the cell;
7. lists the types of mechanisms for removing proteins from the cell;
8. demonstrates the relationship between the structure of ATP and its biological role, and identifies its role for the occurrence of reactions in the cell;
9. presents the characteristic features of the structure of the enzyme;
10. explains the essence of enzyme catalysis;
11. presents the theory of substrate specificity of the enzyme;
12. demonstrates ways to regulate enzyme activity (activation, inhibition);
13. explains the mechanism of negative feedback in the regulation of metabolic pathways (auto-ubiquitination);
14. understands the pathomechanisms of diseases associated with inadequate protein turnover in the cell;
15. presents the tools used in molecular biotechnology (PROTAC compounds, molecular glues) and identifies their applications in medicine;
16. understands the importance of deubiquitinating enzyme inhibitors in medicine.