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Review

P2X7 receptor in normal and cancer cells in the perspective of nucleotide signaling

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Nucleotides are the most common compounds produced constantly by living organisms. They are involved in most cellular processes like the synthesis of other nucleotides and nucleic acids, generation of energy needed for the maintenance of cells, and molecular signaling. In the 70s sir. Geoffrey Burstock discovered a new class of transmembrane proteins – nucleotide receptors responding to nucleotides and their derivatives. For historical reasons, we distinguish two main classes of nucleotide receptors: P1 – which are G protein-coupled adenosine receptors, and P2 – nucleotide receptors that respond to ATP and its derivatives. Additionally, the P2 receptors family can be divided into two subgroups: P2Y – G protein-coupled receptors and P2X cation channel receptors. This paper focuses mainly on the most researched receptor in the nucleotide receptors family – the P2X7 receptor – presenting it in the background of the nucleotide signaling landscape. Almost thirty years of extensive studies on the receptor contributed to understanding protein structure, splicing variants, and mechanism of action in somatic cells. Despite such a wide spectrum of research, the role of the receptor in cancer progression is still undetermined. In many reports, we can find information about the anti-tumorigenic role of this receptor caused by activation of the cell death mechanism after membrane pore formation. These results, however, contradict other studies in which the same receptor is known to promote cancer development through stimulation of proliferation and activation of pro-survival pathways. Ultimately, all this gathered knowledge points to two faces of the receptor in tumor progression. Therefore, we do provide a comprehensive overview of the topic. Finally, we also try to systemize previous and recent literature about the role of this receptor in somatic and cancer cells and provide access to it in the form of a convenient table.

Keywords: P2X7 receptor, nucleotide signaling, cancer, glioma, calcium signaling, cell death

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Abbreviations: ATPγS, Adenosine 5′-O-(3-thio)triphosphate; BBG, Brilliant Blue G (Coomassie brilliant blue); BzATP, 2′(3′)-O-(4-Benzoylbenzoyl)adenosine-5′-triphosphate; CARD, caspase activation and recruitment domain; DAG, 1,2-diacylglycerol; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; IP_3, Inositol trisphosphate; IP3R, inositol trisphosphate receptor; LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide; MIP-1, macrophage inflammatory protein 1; oxATP, oxidized ATP (also oATP); PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC β, phospholipase C β; PLD, phospholipase D; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; SOCE, store-operated calcium entry; TLR4, toll-like receptor 4; TNF-R1, tumor necrosis factor receptor 1; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor

THE ORIGIN OF NUCLEOTIDE SIGNALING

ATP is the most widespread source of chemical energy in metabolic processes. This nucleotide can be found in all cells of plants, animals, and microorganisms. It is constantly produced and consumed in most cellular processes, starting from the synthesis of other nucleotides by the synthesis of nucleic acids and ending with the transport of substances in and out of the cell and the generation of mechanical force. ATP molecules are also used in situations where chemical synthesis reactions require energy input or where regulatory processes require thermodynamic irreversibility of accompanying reactions. The concentration of ATP inside the cell results from a balance between the synthesis of new ATP molecules and their hydrolysis and is strongly related to the metabolic activity of the cell. Typically, in mammalian cells, this value is around 3–5 mM (Jones, 1986; Gorman *et al.*, 2007), thus many orders of magnitude are larger than those in the extracellular space where it is 1–10 nM (Giuliani *et al.*, 2019).

To fully understand the key role of ATP in the development of life on earth, it is necessary to go back to the beginning of the evolution of energy metabolism and the transmission of information using the genetic code. It was in these processes that the first nucleotides played an important part. Inorganic phosphates were the basic building blocks for the development of life on earth. The origin of these compounds on earth is enigmatic, some of the authors postulate even the extraterrestrial origin of phosphates, present on meteorites hitting the earth's surface at an early stage of the planet's development (Bryant *et al.*, 2013). Without these compounds, it would be impossible to create RNA and DNA that still exist to this day and play a key role in the transmission of genetic information. With the development of life, the basic forms of information transmission changed. Initially, there were only long, self-replicating RNA chains in which nucleotides played both a building and energetic role (in the case of nucleic acid synthesis). However, when the first primitive cells appeared and DNA took over the main role of the carrier of the genetic code, nucleotides started to play a role as the energy carrier (Bada, 2004). Over the centuries, two such carriers have emerged: ATP and GTP. ATP participates in the vast majority of reactions while GTP is found in a limited group of them. The prevalence of ATP in living organ-

isms may be associated with its greater free energy of last phosphate bond hydrolysis than in the case of GTP (Denton, 2009; Bazil *et al.*, 2010). Despite the great advantages of using ATP as an energy carrier, this molecule has a serious disadvantage. Due to the chemical properties of ATP, it is not possible to carry out reactions using this molecule in an environment with a high concentration of calcium ions. Combination of calcium and ATP results in forming insoluble salts that cannot be used by living organisms. While the early earth ocean was alkaline, the concentration of calcium ions was low, similar to the present level in the cytoplasm (Plattner & Verkhratsky, 2016). This property of ATP turned out to be disadvantageous when the level of calcium ions on earth significantly increased in the pre-ocean (Kazmierczak *et al.*, 2013). This complication led to the formation of a system of membranes protecting the inside of the cell against an unfavorable environment and a complex system of pumps and exchangers on the surface of these membranes in which ATP played a key energetic role. All these adaptations were to keep the levels of calcium ions low inside the cell. To summarize, ATP is needed to maintain a constant level of calcium in the cell so that ATP can be freely produced and hydrolyzed in it. This dependence made ATP and free calcium ions one of the most basic signaling molecules in the early stages of the evolution of living organisms (Berridge *et al.*, 2003; Clapham, 2007).

As mentioned earlier, there is a very steep gradient of the ATP concentration between the cytoplasm and extracellular medium. Any tissue injury, cell damage, or lysis, including platelet aggregation and thrombosis, will result in a significant local release of ATP. There are also physiological mechanisms of active ATP release, such as connexin hemichannels activity (Anselmi *et al.*, 2008), pannexin channels (Jackson, 2015), and nucleoside transporters (Dos Santos-Rodrigues *et al.*, 2014) or vesicular release inactive synapses (Pankratov *et al.*, 2006). Thus, the presence of extracellular nucleotides created a chance for nucleotide signaling development.

NUCLEOTIDE RECEPTORS

The first report that ATP, apart from its energetic function, can act as a neurotransmitter appeared in 1972 (Burnstock, 1972). Burnstock in his research indicated that the intestines and bladder of a guinea pig subjected to the action of extracellular ATP would contract independently of known neurotransmitters. Initially, the scientific world was skeptical about the idea of nucleotide signaling. This opinion was widespread because of earlier research that focused mainly on the intracellular processes related to the energetic functions of ATP (Gillespie, 1934; Lipmann, 1941; Meyerhof, 1951; Lo *et al.*, 1968). The reluctance of the community was also caused by the results of ATP concentration measurements in the extracellular environment (1–10 nM outside the cell, 3-5 mM in the cytoplasm), and the size and charge of the molecule, which does not allow it to freely penetrate the cell membrane (Chaudry, 1982). In 1976, Burnstock, relying on pharmacological research, defined proteins that participated in the cell's response to extracellular ATP calling them purinergic receptors. In the course of further pharmacological and molecular studies, he divided nucleotide receptors into two groups. Since the signaling role of adenosine was known for half of the century already (Drury & Szent-Györgyi, 1929), the first group was P1 receptors, stimulated by adenosine, and a new group, P2, was created for receptors stimulated by ATP and ADP. Further studies at Burnstock's laboratory led to the division of the P2 family into metabotropic receptors or receptors that evoke a cascade of intracellular reactions as an effect of binding the ligand (Encyclopedia of Pain, 2013) – P2Y – and ionotropic receptors, opening membrane channels after binding the ligand (North, 2016) – P2X (Burnstock & Kennedy, 1985; Burnstock & Verkhratsky, 2012) (Fig. 1). Moreover, studies on the conversion of nucleotides after their release into the extracellular environment have resulted in the discovery of enzymes that hydrolyze nucleotides on the surface of the cell membrane. This created a more comprehensive picture of nucleotide signaling (Ziganshin *et al.*, 1994; Lazarowski *et al.*, 2000).

P1 FAMILY

The receptors stimulated by adenosine are members of the P1 family. They are defined as metabotropic. They belong to a diverse group of G-protein coupled receptors (GPCR). The receptors from this group are made up of seven transmembrane domains. The P1 family of receptors can be divided into four subtypes: A1, A2A, A2B, and A3. The homology between the individual subtypes is approximately 50% (Fredholm *et al.*, 2000). The A1 receptor binds to the Gi/0 proteins and the A2A receptor to the Gs protein. Activation of the A1 receptor reduces the activity of adenylyl cyclase and stimulates the activity of phospholipase C by which there is an increase in the concentration of calcium ions in the cytoplasm (Schulte & Fredholm, 2000; Schulte & Fredholm, 2003). Activation of the A2A receptor increases the activity of adenylyl cyclase and activates signaling pathways related to MAP kinases (Baraldi *et al.*, 2008; Chen *et al.*, 2013). The A2B receptor binds to GsGq/11 proteins, and the A3 receptor binds to GiGq/11 proteins. Activation of A2A increases the activity of adenylyl cyclase, stimulates the activity of phospholipase C, and activates MAP kinases (Sun & Huang, 2016; Bader *et al.*, 2017). The stimulation of the A3 receptor reduces the activity

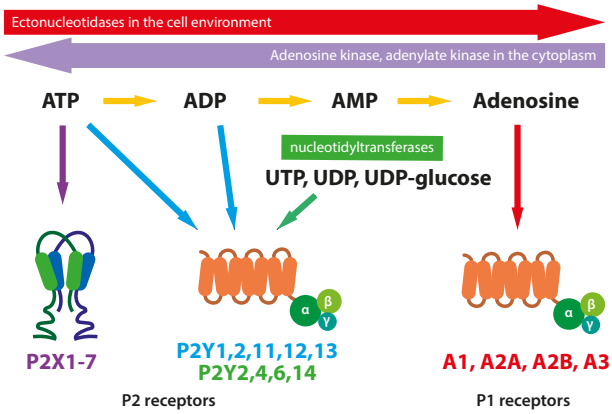

Figure 1. Schematic representation of receptors and ligands engaged in the nucleotide signaling.
The P2 family of receptors respond to ATP, ADP and UTP, and the P1 family to adenosine. P2Y receptors and P1 receptors belong to big family of G-protein coupled receptors, while P2X receptors are membrane channels. The level of nucleotides in the extracellular environment is regulated also by ectonucleotidases, which hydrolyze nucleotides removing phosphate groups. Nucleotidyltransferase transfer phosphate group from one nucleoside to another while adenosine kinase and adenylate kinase add phosphate groups.

of adenylyl cyclase and activates phospholipase C, which, as in the case of the A1 receptor, increases the concentration of calcium ions in the cell. Like all the receptors mentioned above, A3 can stimulate the activity of MAP kinases (Fishman *et al.*, 2002; Borea *et al.*, 2015). A1 and A2A receptors can be stimulated by low adenosine concentrations, while the activation of A2B and A3 receptors is associated with the presence of high adenosine concentrations in the extracellular environment (Borea *et al.*, 2018). P1 family receptors are widely distributed in living organisms and play an important role in maintaining cell homeostasis and are crucial for the development of pathological conditions.

P2 FAMILY

The P2 receptor family groups two completely unrelated subfamilies of nucleotide receptors: metabotropic P2Y receptors and P2X receptors acting as ion channels. The classification of these receptor subfamilies into a common P2 family is related to a historical classification based on pharmacological characteristics and not on a mechanism of action that was unknown at the time. Regarding the ligands that activate them, the P2X and P2Y receptors are similar. However, in terms of the mechanism of action, the P2Y and P2X receptors are completely different.

P2Y receptors

The P2Y family consists of metabotropic receptors activated by extracellular ATP, UTP, and their derivatives. In terms of molecular structure and mechanisms of intracellular signaling, they are GPCR receptors, similar to P1 adenosine receptors.

The P2Y receptor family is one of the most numerous groups of nucleotide receptors. So far, as many as 8 different receptor proteins have been discovered: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14 (Von Kügelgen & Hoffmann, 2016). There are also numerous related orphan receptors, receptors similar in sequence to the family members but without a known ligand (Murakami *et al.*, 2008). An example of such a receptor is the P2Y14 receptor, activated by UDP-glucose, first known for its gene sequence, then characterized biochemically (Abbracchio *et al.*, 2003). This is the most diverse group of nucleotide receptors – the difference in sequence between the individual types ranges from 21% to 57%.

The receptors from the P2Y family are composed of 7 transmembrane domains. The ligand-binding site takes place in the "pocket" formed by the domains TM3, TM6, and TM7 in the cell membrane. The nucleotide affinity for this region is related to positively charged amino acids that exhibit electrostatic interactions with phosphate residues in the nucleotides. The three-subunit (α, β, γ) G proteins are responsible for transmitting signals inside the cell. According to the phylogenetic tree based on differences in gene sequences, P2Y receptors can be divided into two distinct groups. The first includes P2Y1, P2Y2, P2Y4, P2Y6, P2Y11 receptors and the second includes P2Y12, P2Y13, P2Y14 (Schöneberg *et al.*, 2007; Von Kügelgen & Hoffmann, 2016). The first group of receptors is bound to Gq/11 proteins. Their stimulation causes Gq signaling and activation of phospholipase C beta (PLCβ). Activation of this enzyme leads to the hydrolysis of phosphatidylinositol-(4,5)-bisphosphate (PIP2) present in the cell membrane. As a result of hydrolysis, two secondary information transmitters are formed: inositol triphosphate (IP3) and 1,2-diacylglycerol (DAG) (Wypych & Barańska, 2020). Water-soluble IP3 diffuses from the plasma membrane into the cytoplasm and then attaches to inositol trisphosphate receptors (IP3R) on the surface of the endoplasmic reticulum. The IP3 receptors play the role of ion channels that can release calcium ions from the endoplasmic reticulum. This results in an increase in the concentration of calcium in the cytoplasm, and then, as a result of a decrease in the concentration of calcium ions in the endoplasmic reticulum, voltage-independent calcium channels in the cell membrane are opened (Berridge, 1993; Taylor & Thorn, 2001). As a consequence, the concentration of free calcium ions in the cell increases through the influx of ions from the intercellular space. This process of secondary calcium influx from the extracellular space following the emptying of calcium resources in the endoplasmic reticulum is called Store Operated Calcium Entry (SOCE) (Hogan & Rao, 2015). The second of the secondary transmitters caused by PLCβ activity, DAG, remains in the plasma membrane and activates protein kinase C (PKC). Active PKC can have an inhibitory effect on the receptor itself and PLC by inhibiting PIP2 hydrolysis and reducing the strength of the calcium signal. In addition, activation of PKC leads to an increase in the activity of phospholipase D (PLD) and the MAP kinase pathway (Wypych & Barańska, 2020). In summary, the activation of P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 receptors leads to the mobilization of calcium ions in the cell. However, it should be remembered that GPCR receptors do not show 100% specificity for the binding of the α subunits of G proteins, and so in the case of the P2Y11 receptor, alternative activation may occur related to the binding of the Gs protein, which leads to an increase in the activity of adenylyl cyclase and, consequently, an increase in the level of cAMP in the cell (Kennedy, 2017). The affinity of G proteins can also be altered after P2Y receptors complex with other membrane proteins. An example is the complex of integrin αvβ5 with the P2Y2 receptor, under these conditions the receptor can interact with the G0 protein activating signal transduction by the Rac1 protein. Moreover, the conformational change of the P2Y2 receptor by integrin αvβ5 causes signal transduction through the G12 protein and ROCK kinase (Kłopocka *et al.*, 2020). The P2Y12, P2Y13, and P2Y14 receptors belonging to the second group reduce the level of cAMP in the cell by inhibiting the activity of adenylyl cyclase. In these receptors, intracellular signal transduction is mediated by the Gi/0 protein.

P2X FAMILY

Receptors from the P2X family are completely unrelated to P2Y receptors. P2X receptors are non-selective cation channels that open upon stimulation with ATP or its synthetic analogs (North, 2016), causing ion current flow and depolarization of the cell membrane. Such receptors are called ionotropic receptors. The ionotropic receptors are not monomers – for the receptor to function properly, a multi-molecular, oligomeric protein complex must be formed, which creates an ion channel in the cell membrane. These receptors can form both homo- and heterooligomers. A functional P2X receptor usually consists of three subunits from which the ion channel is created (Ralevic & Burnstock, 1998). The P2X receptor subunit consists of two transmembrane regions (TM1 and TM2), intracellular C- and N-terminals, and an extracellular loop that can undergo many post-trans-

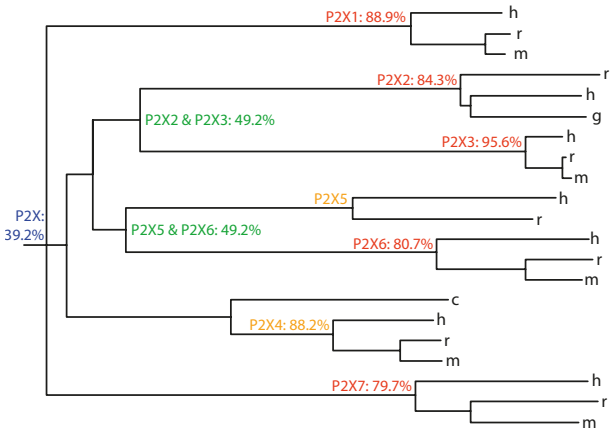

Figure 2. Cladogram of the relationships between P2X family members in the form of a phylogenetic tree.
The percentages show the degree of amino acid chain similarity between the individual receptors. The abbreviations stand for: h, human receptor protein; r, rat receptor protein; m, mouse receptor protein; g, guinea pig receptor protein; c, chicken receptor protein. Modified from (North & Surprenant, 2000).

plantation modifications. The TM1 transmembrane domain is involved in the regulation of the conduction of channels, while the TM2 domain plays a key role in ion channel formation (Khakh *et al.*, 2001). In the extracellular loop, there are localized binding sites for ATP and allosteric regulators (Garcia-Guzman *et al.*, 1997; Clarke *et al.*, 2000; Jiang *et al.*, 2000a; Ennion *et al.*, 2000). So far, seven genes encoding subunits of the P2X family of receptors have been discovered, numbered 1 to 7 (North, 2002). The similarity between the proteins in this family ranges from 30 to 50 percent depending on the receptor (Fig. 2). P2X family receptors are widely distributed in mammalian cell lines and tissues (Burnstock & Knight, 2004; Burnstock, 2007).

P2X7 receptor

The P2X7 receptor stands out from the P2X family. The first reports of this receptor's existence date back to 1980, when Cockcroft and Gomperts observed increased histamine secretion from rat mast cells upon stimulation with high concentrations of extracellular ATP (Cockcroft & Gomperts, 1980). In 1986 Gordon put forward a theory, based on data from pharmacokinetic analyzes, of the presence of a new P2Z receptor that can be activated with low doses of synthetic agonists or high ATP concentrations compared to other nucleotide receptors (Gordon, 1986). In addition, stimulation of the P2Z receptor resulted in massive membrane depolarization and the formation of a pore in the cell membrane, permeable to molecules below 900 kDa (Ferrari *et al.*, 1996; Coutinho-Silva *et al.*, 1996). The identification of the new receptor took place in 1996 when the surprenant research group successfully cloned the rat P2X7 gene and reestablished its activity in human HEK293 cells (Surprenant *et al.*, 1996). Since the publication of the above work, research on this type of receptor has begun in other species of animals. The receptor was classified into the P2X family (Rassendren *et al.*, 1997).

STRUCTURE OF THE GENE ENCODING P2X7

The gene encoding the P2X7 receptor is commonly found in all mammals and other vertebrates (Donnelly-Roberts *et al.*, 2009). The human gene encoding the human P2X7 receptor (hP2X7) is located on chromosome 12 at locus q24. This human gene is composed of 53 733 base pairs of 13 exons, which can create 10 different variants of gene splicing (Buell *et al.*, 1998). The structure of the receptor gene in mice shows 81% similarity to the human gene encoding this receptor. The gene encoding the mouse P2X7 receptor is located on chromosome 5 at locus 62.5 cM (Chessell *et al.*, 1998). The gene encoding the rat P2X7 protein is located on chromosome 12 at locus q16 and shows 80% homology to the human gene (Surprenant *et al.*, 1996). The P2X7 gene in all species studied so far consists of 13 exons.

The regulation of P2X7 gene transcription can take place with the participation of promoters and enhancers of transcription, microRNA, and long coding RNA. So far, the structure of human and mouse promoters has been studied. The promoter region of the human P2X7 gene is located between nucleotides –158 to +38 flanking the transcription initiation region (Zhou *et al.*, 2009). Additionally, the transcription of the human P2X7 gene may be regulated by unknown transcription enhancers at the +222 – +323 and +401 – +573 sites by cytosine hypermethylation. The promoter region of the gene encoding the mouse P2X7 receptor is located between nucleotides –249 to +17 surrounding the transcription start site (García-Huerta *et al.*, 2012). Additionally, transcription enhancers responsive to stimulation by retinol (vitamin A) have been identified in the mouse gene (Heiss *et al.*, 2008; Hashimoto-Hill *et al.*, 2017). Interestingly, in studies on the human P2X7 receptor, an opposite effect was observed. The administration of retinol decreased the level of the P2X7 receptor in cells of neuronal origin in humans (Wu *et al.*, 2009; Orellano *et al.*, 2010).

P2X7 mRNA can also be efficiently regulated by miRNA (microRNA) molecules. The miR-186 molecule can lower the P2X7 transcript level in podocytes in the kidney (Sha *et al.*, 2015). On the other hand, the miR-150 molecule in mice lowers the level of P2X7 receptor in the lung epithelium, while regulating the level of surfactant secretion by these cells (Weng *et al.*, 2012). Moreover, miR-150 may have a cardioprotective effect on cardiomyocytes in a mouse model of cardiac ischemia (Tang *et al.*, 2015) by regulating the apoptosis of damaged cells. Regulation of P2X7 levels by microRNA molecules is also possible in cancer cells. The miR-150, miR-186, and miR-21 molecules that lower the P2X7 transcript are overproduced in breast, cervical, bladder, and lung cancers (Zhou *et al.*, 2008; Huang *et al.*, 2013; Boldrini *et al.*, 2015). Additionally, the P2X7 receptor after stimulation can cause the release of microvesicles and exosomes from melanoma cells containing mRNA (Pegoraro *et al.*, 2021a).

The last known possibility of regulating the transcription level of the P2X7 gene is by long-noncoding RNA (lncRNA). Small interfering RNAs complementary to lncRNA NONRATT021972 lower P2X7 receptor levels in many pathological conditions such as neuropathic pain (Liu *et al.*, 2016), myocardial hypoxia (Zou *et al.*, 2016; Tu *et al.*, 2016), and under metabolic stress (Xu *et al.*, 2016; Li *et al.*, 2016a). siRNA complementary to lncRNA uc.48+ reduces the level of P2X7 receptors in neurons of diabetic rats (Wu *et al.*, 2016).

THE PROTEIN STRUCTURE OF THE P2X7 RECEPTOR

A functional P2X7 receptor, like all P2X family receptors, is composed of at least three subunits encoded by

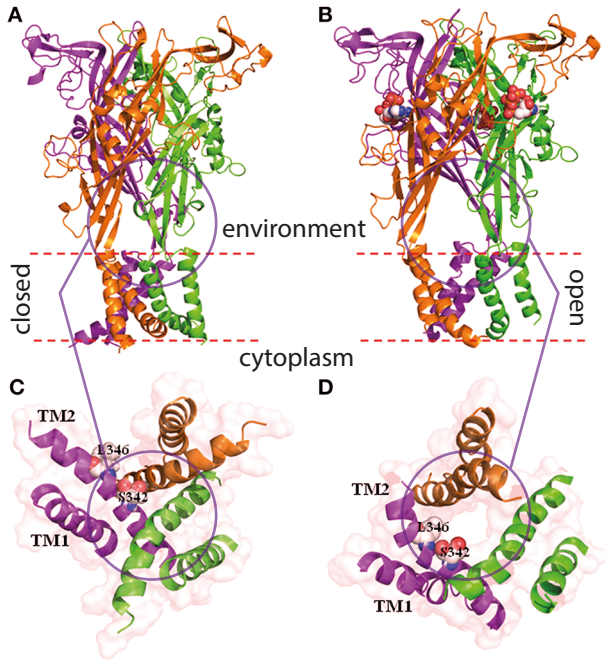

Figure 3. Structure of the functional trimer P2X7.
(A, C) P2X7 receptor in an unstimulated state with a closed ion channel. (**B** and **D**) P2X7 receptor stimulated by extracellular ATP with an open ion channel modified according to (Wei *et al.*, 2016, on Creative Commons Licence CC BY 4.0).

the P2RX7 gene. In the structure of the subunit, two transmembrane domains, the extracellular loop (with ATP binding sites) and the intracellular N- and C-terminus, can be distinguished. N-terminus regulates the flow of calcium ions through the ion channel and is responsible for the activation of signal kinases (Amstrup & Novak, 2003; Liang *et al.*, 2015) (Fig. 3). The C-terminus of the P2X7 receptor is significantly longer than that in other P2X family receptors. It is postulated that this part is responsible for the oligomerization of the receptor protein and pore formation (Kim *et al.*, 2001; Smart *et al.*, 2003; Costa-Junior *et al.*, 2011; Kopp *et al.*, 2019). Moreover, there is also a binding site for lipopolysaccharide at the C-terminus, which demonstrates the important role of this receptor in the regulation of inflammatory processes (Denlinger *et al.*, 2001; Leiva-Salcedo *et al.*, 2011). Additionally, this terminus contains domains that can interact with other proteins (Surprenant *et al.*, 1996).

Post-translational modifications of the P2X7 receptor

The P2X7 receptor protein can undergo many post-translational modifications such as phosphorylation/dephosphorylation, N-glycosylation, palmitoylation, and ADP-ribosylation.

There are several putative sites at the C-terminus of the receptor where phosphorylation may occur (Y382-384). Studies on the phosphorylation pattern of this region in human microglia have shown a significant role of this receptor in the regulation of pain and tolerance to opioids (Leduc-Pessah *et al.*, 2017). An interesting fact is that these modifications did not affect the ion permeability of the P2X7 receptor channel. The C-terminus of the P2X7 receptor can also be palmitoylated. This modification is associated with interactions between the receptor and the cell membrane. The targeted mutagenesis of the palmitoylation site influenced the presence of the receptor in the plasma membrane. Cells with a mutant P2X7 receptor variant incapable of palmitoylation had decreased levels of this receptor in the plasma membrane but high levels in the endoplasmic reticulum (Gonnord *et al.*, 2009).

The region which undergoes N-glycosylation is the extracellular loop. Five places can undergo this modification: N187, N202, N213, N241, and N284. The pattern of glycosylation may vary depending on the type of tissue (Lenertz *et al.*, 2010). N-glycosylation, in addition to affecting the level of the receptor in cells, also causes changes in signal transduction inside the cell. Site-directed mutagenesis at N187 blocked modification of this site, resulting in decreased P2X7 receptor activity (Di Virgilio *et al.*, 2018).

The last known post-translational modification that the P2X7 receptor can undergo is ADP-ribosylation. This modification causes an alternative, permanent activation of P2X7 by mimicking the attachment of ATP in the active site. ART2.1 and ART2.2 enzymes ribosylate the P2X7 receptor at site R125, at the hypothetical ATP binding site, resulting in its constant activation and a decrease in the affinity for extracellular ATP (Adriouch *et al.*, 2001, 2008). This modification has only been observed in rodents as the ART2.1 and ART2.2 enzymes needed for this process are not present in human tissues (Haag *et al.*, 1994).

P2X7 protein splicing variants

So far, 10 splicing variants of the gene encoding the human P2X7 receptor have been identified (Pegoraro *et al.*, 2021b) (Fig. 4). The variant that is responsible for the full and functional structure of the receptor subunit was named P2X7A, while the subsequent variants resulting from alternative splicing were named with the letters B to J. However, the conducted studies showed that only 3 of the truncated protein variants can form receptor subunits. These are P2X7B, P2X7H, and P2X7J (Feng *et al.*, 2006; Adinolfi *et al.*, 2010; Giuliani *et al.*, 2014). The P2X7B isoform is characterized by a short carboxyl fragment compared to the P2X7A isoform. This isoform, 364 amino acids long, forms a functional ion channel which, due to the truncated carboxyl fragment, cannot form a functional pore in the cell membrane. Long-term stimulation with P2X7B does not lead to cell death and promotes the formation of pores in the cell membrane. Moreover, the presence of this isoform may intensify proliferation by stimulating the NFAT1 transcription factor with calcium ions (Adinolfi *et al.*, 2010). Another isoform – P2XH with a length of 274 amino acids – forms a non-functional ion channel (Cheewatrakoolpong *et al.*, 2005). The last of the receptor isoforms detected at the

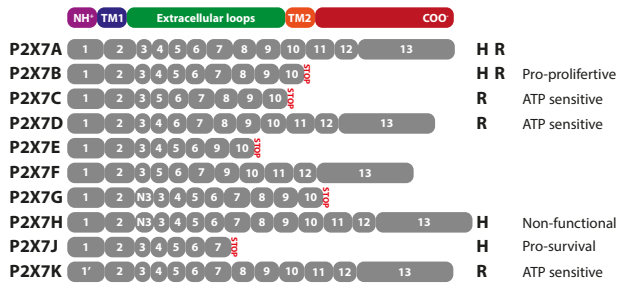

Figure 4. Graphical representation of possible alternative splicing variants of the P2X7 receptor.
R, isoform from rodents; H, human isoform. Modified according to (Andrejew *et al.*, 2020).

protein level is the P2X7J isoform. It is composed of 258 amino acids and can form, together with the P2X7A isoform, non-functional heterotrimers. Such a heterotrimer increased the resistance of cervical cancer cells to extracellular ATP without causing cell death (Feng *et al.*, 2006).

In rodents, four splice variants were also detected for the gene encoding the P2X7 subunit (P2X7B, P2X7C, P2X7D, P2X7K) along with the canonical P2X7A isoform composed of 595 amino acids. The P2X7B and P2X7D isoforms can form heterodimers with the P2X7A isoform, reducing the activity of the P2X7 receptor constructed in this way. The P2X7K isoform, composed of 592 amino acids, after the creation of the P2X7 receptor has a greater affinity for ATP than the receptor composed of subunits of the P2X7A isoform (Schwarz *et al.*, 2012).

This clear image is somehow obscured by the existence of nfP2X7 form, defined by the binding of the specific antibody, raised against amino acid sequence 200-216, with particular conformation present in tumor cells P2X7 receptor (Gilbert *et al.*, 2019). However, the mechanism of the epitope presentation is unknown, and we do not know if nfP2X7 is another isoform produced in certain circumstances or some modification of the wild protein. It was shown that nfP2X7 is widely present both in cells able to open P2X7-dependent pore as well as those unable to open membrane pore, its presence was confirmed in prostate cancer cells (LNCaP, PC3, DU 145), KELLY neuroblastoma, Ramos (RA-1) lymphoma. Antibodies against nfP2X7 passed the first stage of clinical trials in basal cell carcinoma (Gilbert *et al.*, 2017).

ACTIVATION OF THE P2X7 RECEPTOR

The P2X7 receptor, like all receptors in this group, is activated upon stimulation with extracellular ATP. However, compared to other P2X receptors, the ligand concentration needs to be higher, from 50 μM to 2.5 mM. The concentration that effectively activates the receptor varies from species to species (Surprenant *et al.*, 1996; Rassendren *et al.*, 1997; Fonfria *et al.*, 2008; Roman *et al.*, 2009; Bradley *et al.*, 2011).

The P2X7 receptor can also be activated by synthetic ATP analogs such as 2(3)-O-(4-benzoylbenzoyl) ATP (BzATP) and adenosine 5-(γ-thio) triphosphate (ATPγS). BzATP has about 10 times greater affinity for the P2X7 receptor than ATP. The exception is that the P2X7 receptor derived from guinea pig (Fonfria *et al.*, 2008). In this case, BzATP activates the P2X7 receptor only slightly stronger than ATP itself. ATPγS activates P2X7 originating from mice, rats, dogs, and humans. What is important, the level of P2X7 activation with ATPγS is significantly lower compared to the P2X7 activation with BzATP and ATP (Donnelly-Roberts *et al.*, 2009; Spildrejorde *et al.*, 2014) (Table 1).

An interesting and characteristic of P2X7 activation is its regulation by lipopolysaccharide from gram-negative bacteria (LPS). Inserting a genetic construct inside the cell that causes the production of LPS in the cytoplasm, stimulates caspase 11 that activates the pannexin-1 channel by cleaving. This results in increased release of ATP into the extracellular environment and activation of P2X7 (Yang *et al.*, 2015). Moreover, it is postulated that LPS may increase the sensitivity of the P2X7 receptor to extracellular ATP by binding to the cytoplasmic LPS binding domain in this receptor and conformational changes (Denlinger *et al.*, 2001).

Reports are indicating a positive role of cathelicidins in the regulation of P2X7 receptor activity. The antibacterial LL-37 peptide belonging to this family activated the P2X7 receptor, causing the maturation and release of IL-1β (Elssner *et al.*, 2004). Studies by other research groups, however, indicate that LL-37 does not activate the P2X7 receptor directly but acts as an allosteric ATP sensitivity enhancer (Pochet *et al.*, 2006; Tomasinsig *et al.*, 2008). The activity of P2X7 can be regulated similarly by numerous substances such as tenidap (Sanz *et al.*, 1998), polymyxin B (Ferrari *et al.*, 2004), clemastine (Nörenberg *et al.*, 2011), ivermectin (Nörenberg *et al.*, 2012) and ginsenosides (Helliwell *et al.*, 2015). The positive allosteric modulator of P2X7 was successfully employed in cancer with its ability to negatively control the growth and development of lung tumor by potentiating αPD-1 treatment (Douguet *et al.*, 2021).

P2X7 RECEPTOR INHIBITION

During intensive research on the P2X7 receptor, many chemical compounds were developed that could inhibit its activity. Each of these antagonists differs in receptor affinity, mechanism of action, and species specificity.

The first generations of P2X7 inhibitors were not very specific – apart from inhibiting, P2X7 also affected the activity of other P2 family receptors (Gever *et al.*, 2006). Examples of such inhibitors are PPADS (pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid) (Lambrecht *et al.*, 1992; Valera *et al.*, 1994) and suramin (Urbanek *et al.*, 1990), which blocked also other receptors from the family: P2X, P2Y, and P1. A much more specific inhibitor of the first generation P2X7 was oxi-

Table 1. Summary of the activity of P2X7 receptor agonists depending on the species and agonist used.
Based on (Bartlett et al., 2014).

Species	Sequence identity (%)	EC_{50} (μM)	
		ATP	BzATP
Human (*Homo sapiens*)	100	96	5
Rhesus macaque (*Macaca mulatta*)	96	800	58
Dog (*Canis familiaris*)	85	1148	21
Rat (*Rattus norvegicus domestica*)	80	85	4
Mouse BALB/c (*Mus musculus*)	80	200	60
Mouse C57BL/6 (*Mus musculus*)	80	162	36
Guinea pig (*Cavia porcellus*)	77	603	>200

dized adenosine triphosphate (oxATP) (Murgia *et al.*, 1993), which binds irreversibly to the P2X7 receptor. However, further research has shown that it is not an ideal antagonist. In addition to the irreversible inhibition caused by covalent changes in the protein structure, oxATP can also inhibit the activity of P2X2 and P2X3 receptors (Evans *et al.*, 1995).

One of the most widely used first-generation P2X7 inhibitors is Brilliant Blue G (BBG), also known as the Coomassie brilliant blue (Soltoff *et al.*, 1989; Jiang *et al.*, 2000b). This inhibitor has the greatest affinity for the rat P2X7 receptor, blocking it at nanomolar concentrations. It is a relatively cheap and non-toxic inhibitor; however, it should be mentioned that it is not without its drawbacks. BBG can inhibit the activity of other P2X receptors such as P2X1, P2X2, P2X3, and P2X4 (Jiang *et al.*, 2000b). Moreover, there are reports of pannexin-1 inhibition by BBG (Qiu & Dahl, 2009; Dahl *et al.*, 2013). It is also worth mentioning that BBG is a dye that can cause problems in some applications.

With the development of new screening methods and the understanding of the exact protein structure of the P2X7 receptor, second-generation inhibitors have emerged. Their development was a result of the growing attention in the pharmaceutical industry, which resulted from the recognition of the important role of the P2X7 receptor in the regulation of inflammation (Di Virgilio *et al.*, 2017) and neuropathic pain (Zhang *et al.*, 2020). The second P2X7 antagonists also had the ability to cross the blood-brain barrier, which, apart from the first-generation inhibitor – BBG – had not been possible before (Peng *et al.*, 2009; Apolloni *et al.*, 2021). The first-generation P2X7 inhibitors were mainly based on *in vitro* studies and were found to be often unsuitable for *in vivo* use. This was due to the rapid degradation of the inhibitor after administration to the animal and its poor pharmacokinetic parameters (Bartlett *et al.*, 2014). New generation inhibitors, which include molecules such as CAY10593, A-839977, AACBA hydrochloride, and 1-Benzyl-5-aryltetrazoles, are characterized by increased efficiency compared to the first generation and greater bioavailability in the *in vivo* systems (Nelson *et al.*, 2006; Michel *et al.*, 2007; Broom *et al.*, 2008; Honore *et al.*, 2009; Letavic *et al.*, 2013; Pupovac *et al.*, 2013).

Alternatives to inhibitors in the form of small chemical molecules are monoclonal antibodies and single-domain antibodies (sdAb, nanobodies) directed against the P2X7 receptor. Administration of the anti-P2X7 antibody reduced inflammation in a mouse model of ulcerative colitis (Kurashima *et al.*, 2012). On the other hand, the use of nanobodies directed against P2X7 reduced allergic contact dermatitis in an *in vivo* mouse model (Danquah *et al.*, 2016). Anti-P2X7 antibodies can also bind to short or non-functional isoforms of the receptor (Barden *et al.*, 2003). The use of the anti-P2X7 antibody decreased the activity of the P2X7 receptor in murine B16 melanoma cells resulting in a reduction in tumor size (Gilbert *et al.*, 2017).

The inhibition of the P2X7 receptor may also be influenced by the extracellular ion concentration. A high concentration of magnesium ions Mg^{2+} can reduce channel activity upon ATP stimulation in rat and human cells (Rassendren *et al.*, 1997). Further studies of this phenomenon showed the influence of other ions such as calcium, zinc and copper on the activity of P2X7. The concentration of hydrogen ions (pH) also had a significant effect on the P2X7 activity (Virginio *et al.*, 1997).

FUNCTIONS AND PHYSIOLOGY OF THE P2X7 RECEPTOR IN NORMAL CELLS

Regulation of interleukin secretion to the extracellular environment

One of the most extensively studied molecular functions of the P2X7 receptor is its participation in the development of inflammation. More specifically, P2X7 takes part in the formation of the NLPR3 inflammasome, and then in the maturation and release of interleukin 1β to the extracellular environment by macrophages and other cells of the immune system (Gudipaty *et al.*, 2003; He *et al.*, 2013). Interleukin 1β is one of the most studied pro-inflammatory interleukins. It takes an active part in the defense of the cell against pathogens and in many metabolic, autoimmune and cancer diseases (Dinarello, 1994, 2018; Macarthur *et al.*, 2004).

The secretion of interleukin takes place in two stages. In the first stage, pro-interleukin 1β and components of the NLPR3 inflammasome are synthesized by stimulation of the transcription factor NF-κβ. This factor is activated when the cell is exposed to specific pathogen-associated molecular patterns (PAMP). An example of such a pattern is extracellular LPS, which by combining with lipopolysaccharide-binding protein (LBP) and CD14 protein forms complexes activating the toll-like receptor 4 – TLR4. The second stage involves inflammasome assembly, caspase 1 activation, and the release as well as the maturation of interleukin 1β. Extracellular ATP activates the P2X7 receptor located on the outer membrane of the immune system cells, acting as a non-selective ion channel and causing the penetration of calcium (Ca^{2+}) and sodium (Na^{+}) ions into the cell interior following the concentration gradient. At the same time, potassium (K^{+}) ions are released from the cell following the concentration gradient, which is a key phenomenon in the production of interleukin 1β (Perregaux & Gabel, 1994; Muñoz-Planillo *et al.*, 2013). A sudden decrease in the level of potassium in the cell causes the activation of caspase 1 (Kahlenberg & Dubyak, 2004). An adapter molecule ASC (apoptosis-associated speck-like protein containing a CARD), which has a CARD (caspase activation and recruitment domain) domain, binds to the overproduced in the first stage NLPR3 protein. This domain enables enhanced caspase 1 activation by cleavage of pro-caspase. The active caspase performs the proteolytic cleavage of the immature form of IL-1β from the 35 kDa form to the 18 kDa active form (Gross *et al.*, 2011). The altered cytokine is released from the cell inducing inflammation.

Experiments with mice lacking the gene encoding the P2X7 receptor demonstrated its key role in the formation of the NLPR3 inflammasome (Solle *et al.*, 2001). These mice showed decreased levels of IL-1β and IL-6 production after ATP stimulation, which may indicate the role of the P2X7 receptor in the pro-inflammatory response.

The role of the P2X7 receptor in other pro-inflammatory pathways

The P2X7 receptor is also involved in the secretion of prostaglandins into the extracellular environment. In murine macrophages, stimulation of the P2X7 receptor results in the secretion of prostaglandin E2, thromboxane B2, and leukotriene B4 (Barberà-Cremades *et al.*, 2012). Activated P2X7 may also affect the hydrolysis of arachidonic acid, which is a substrate for the synthesis

of cyclooxygenase, and prostaglandin E2, which together play an important role in inflammation, fever, and pain (El Ouaaliti *et al.*, 2012). Moreover, P2X7 activation is observed in damaged tissues where the inflammatory infiltration begins. Extracellular ATP stimulation of murine Kupffer cells induced necrotic death of these cells and the release of large amounts of prostaglandin E2 and IL-1β (Toki *et al.*, 2015). The above studies indicate P2X7 as an interesting target for the treatment of inflammation that could be an alternative to cyclooxygenase inhibitors (Barberà-Cremades *et al.*, 2012).

Stimulation of the production of reactive oxygen and nitrogen species

Reactive oxygen species (ROS) are a common signaling element in a living cell. The enzymes of the respiratory chain, present in every actively metabolizing cell, while conducting oxidative phosphorylation produce large amounts of reactive oxygen and nitrogen species (Hoffman & Brookes, 2009), normally reduced by antioxidative systems (Bae *et al.*, 2011). Reactive oxygen species produced by macrophages and microglia play a protective role against microbial invasion.

In many pathological conditions such as cancer or metabolic diseases, the level of free radicals is much higher than in normal cells (Liou & Storz, 2010; Alfadda & Sallam, 2012; Galadari *et al.*, 2017). Nucleotide signaling can stimulate the production of reactive oxygen species. The P2X7 receptor in microglia and macrophage cells by influencing the phosphorylation of NADPH oxidase increases the production of reactive oxygen species. The molecular mechanism of this activation is unclear, but it is known from the literature that NADPH oxidases can be activated by many pathways related to calcium signaling, for example, protein kinase C, mitogen-activated protein kinases p38 and ERK1/2, and phosphatidylinositol 3-kinase (Guerra *et al.*, 2007; Martel-Gallegos *et al.*, 2013).

It has been observed that the continuous stimulation of the P2X7 receptor by low ATP concentrations does not cause cell death but increases the polarization of the mitochondrial membrane through an increased level of free calcium ions in the cytoplasm and thus also in the mitochondria. This calcium influenced oxidative phosphorylation, which stimulated cellular metabolism and influenced cell survival in conditions of extracellular glucose deprivation (Adinolfi *et al.*, 2005; Amoroso *et al.*, 2012).

Creating a cell membrane pore

The unique property of the P2X7 receptor, which distinguishes it from all other receptors in the P2X family, is its dual nature. In addition to the non-selective cation channel, activated P2X7 can induce the formation of a membrane pore, the opening of which leads to the penetration of large molecules (above 900 kDa) and cell death (Surprenant *et al.*, 1996; Rassendren *et al.*, 1997). With prolonged stimulation with P2X7 agonists, the penetration into the cell of organic cations such as N-methyl-D-glucamine increases (Jiang *et al.*, 2005), a cation that is much larger than calcium, potassium, or sodium ions. The time of pore formation varies and depends on many factors, such as the type of cell line, the splice variant of the P2X7 receptor protein, and the agonist with which it is stimulated.

So far, two potential mechanisms of cell pore formation following stimulation of the P2X7 receptor have been presented. The first is associated with a protein that directly interacts with the P2X7 receptor - Pannexin-1. Many reasons point to the crucial role of this protein in the formation of the cell pore (Monif *et al.*, 2009; Suadicani *et al.*, 2012). Experiments with small interfering RNA, complementary to the gene encoding Pannexin-1, showed that lowering the level of this protein in the THP-1, 1321 N1 and J774 cell lines significantly inhibited the penetration of large fluorescent molecules after P2X7 receptor stimulation (Pelegrin & Surprenant, 2006; Locovei *et al.*, 2007; Iglesias *et al.*, 2008). Moreover, the credibility of this theory is confirmed by experiments using the co-immunoprecipitation technique, which showed a direct interaction of the P2X7 receptor with Pannexin-1 (Li *et al.*, 2011; Poornima *et al.*, 2012).

Experiments using murine macrophages with a deleted gene encoding Pannexin-1 suggest the existence of other mechanisms. These cells, despite the lack of Pannexin-1, showed no reduction in the efficiency of penetration of dyes into the cell interior (Qu *et al.*, 2011; Lemaire *et al.*, 2011).

Moreover, in HEK293 cells with a constant, increased level of the P2X7 receptor, penetration of only cationic dyes such as Yo-Pro-1 and ethidium bromide was observed. In murine macrophages, penetration of cationic dyes through the pore was observed while anionic dyes penetrated by diffusion (Schachter *et al.*, 2008; Cankurtaran-Sayar *et al.*, 2009).

Effect of P2X7 receptor activation on cell membrane reorganization

The P2X7 receptor is one of the many proteins involved in the organization of the cell membrane structure. The mechanism of this activity is poorly understood, but the stimulation of extracellular ATP in some cell types may lead to the formation of numerous vesicles and bulges in the cell membrane, shortly after the agonist administration (MacKenzie *et al.*, 2001). Several candidates are postulated as the proteins responsible for changes in the cell membrane. The Rho-associated protein kinase 1 (ROCK1) (Morelli *et al.*, 2003) is suspected to be a regulator behind this effect. The heat shock protein HSP90 is an inhibitor of the effects observed in the cell membrane (Adinolfi *et al.*, 2003). The EMP2 protein interacts directly with the C-terminus of the P2X7 receptor, which may indicate a link between changes in the structure of the cell membrane and the formation of the cell pore following stimulation of the P2X7 receptor (Wilson *et al.*, 2002). Expression of the P2X7 receptor is also observed in leukocyte cells where it is postulated to be responsible for the reorganization of the cell membrane prerequisite for cell migration through blood vessels and the extracellular matrix (Qu & Dubyak, 2009). Moreover, the appearance of vesicles in the plasma membrane following P2X7 receptor stimulation is considered to be the first step in the formation of extracellular vesicles. The activated release of microvesicles and exosomes is a result of the P2X7 stimulation with a high concentration of ATP (Lombardi *et al.*, 2021; Vultaggio-Poma *et al.*, 2022). The triggered release of the extracellular vesicles was not only observed in the immune and central nervous system cells but also in the melanoma cells, which indicates the pro-metastatic activity of the P2X7 receptor (Pegoraro *et al.*, 2021a).

Regulation of cell death

It is not surprising that the P2X7 receptor is involved in the regulation of cell death since one of the recep-

tor's domains localized at the C-terminus is similar to the death domain in the tumor necrosis factor receptor 1 (TNF-R1), which is involved in the induction of apoptosis (Zanovello *et al.*, 1990; Chow *et al.*, 1997; Denlinger *et al.*, 2001). Initially, activation of this receptor was thought to lead to necrotic cell death (Di Virgilio *et al.*, 1989). However, further research has shown that P2X7 is involved in other types of cell death. In the literature, we can find information about the induction of pyroptosis, a form of lytic programmed cell death (Yang *et al.*, 2015), necroptosis, a programmed form of necrosis (Dubyak, 2012), and autophagy (Young *et al.*, 2015). There are also documented cases of characteristic markers of different types of cell death within the same cell. In ATP-stimulated mouse lymphocytes, cell shrinkage was first observed, which is characteristic of apoptotic death, and then after a few minutes, the treated cells disintegrated as in necrotic death (Taylor *et al.*, 2008).

The ability to activate cell death depends on the splice variant of the receptor present in the affected cell. Activation of the P2X7B receptor variant did not result in cell death in transfected HEK293 cells (Adinolfi *et al.*, 2010).

In conclusion, the great majority of the P2X7 receptor does not induce apoptotic death. The most common type of cell death when the P2X7 receptor is activated is necrosis. Moreover, receptor activation does not always lead to any cell death – it depends on the type of cells and the receptor isoform (Di Virgilio *et al.*, 2017).

Stimulation of cell division

As mentioned in the previous section, the P2X7 receptor has long been recognized as a factor leading to cell death (Di Virgilio *et al.*, 2017). However, intensive research using various cell lines and receptor assembly variants has shown that P2X7 may also play a completely different role. The first experiments with lymphocytes transiently transfected with P2X7 receptor showed increased proliferation of these cells upon ATP stimulation (Baricordi *et al.*, 1999). In T cells, P2X7 receptor activation stimulated autocrine secretion of ATP and interleukin-2 into the environment. IL-2 stimulated the activation of transcription factors in T lymphocytes, which as a result increased their proliferation intensity in BALB/c mice (Yip *et al.*, 2009). Studies on alternative receptor splicing variants have suggested that it is the shorter P2X7B isoform lacking the C-terminal fragment that increases the intensity of proliferation (Adinolfi *et al.*, 2005, 2010). Additionally, experiments conducted with the use of microglial cells showed the significant role of the P2X7 receptor in stimulating cell proliferation. Inhibition of P2X7 activity by inhibitors or derivation of a cell line with a mutant receptor variant (G345Y) significantly decreased the intensity of microglial proliferation (Bianco *et al.*, 2006; Monif *et al.*, 2009; Monif *et al.*, 2010).

FUNCTIONS OF P2X7 IN CANCER CELLS

The P2X7 receptor is as common in cancer cells as it is in somatic cells. Stress-related to intra-tumor hypoxia and cancer cell death as a result of chemotherapy or radiotherapy (Martins *et al.*, 2009; Lecciso *et al.*, 2017) may increase the release of ATP into the environment. In addition, the presence of tumor-specific proteins such as SLC29A1, SLC29A2, and SLC28A1-A3 nucleoside transporters may significantly affect the increased concentration of ATP in the tumor environment (Gray *et al.*, 2004; Young *et al.*, 2013). The observed effect of the increased concentration of extracellular nucleotides on cancer depends, however, on the type of tissue from which the tumor originates. Therefore, it should be described in some detail how different the P2X7 receptor response may be depending on the cancer type. The chapter is summarized in Table 2.

P2X7 in prostate cancer

Developing prostate cancer is characterized by a high expression of mRNA encoding the P2X7 receptor as compared to normal tissue (Ravenna *et al.*, 2009). Immunohistochemical studies, using fragments of postoperative prostate tumors from 116 different cases, demonstrated a high level of the P2X7 receptor protein (Slater *et al.*, 2004a). Moreover, the level of this protein has correlated with high expression of prostate-specific antigen (PSA) which is known and widely used as a marker of prostate cancer malignancy (Slater *et al.*, 2005). These reports indicated that the level of the P2X7 receptor could be used as a negative prognostic factor in the development of this type of cancer. It was also noticed that the level of the P2X7 receptor protein correlates with the level of receptors supporting cell division, such as the epidermal growth factor receptor (EGFR) and the estrogen receptor alpha (ERα), which may indicate the significant roles of P2X7 in promoting a more malignant tumor (Ravenna *et al.*, 2009). In addition, functional *in vitro* studies using prostate cancer cell lines showed increased aggressiveness and invasiveness of these lines after stimulation of the P2X7 receptor by extracellular ATP (Ghalali *et al.*, 2014; Qiu *et al.*, 2014). Moreover, in the PC-3M human prostate carcinoma cell line where functional P2X7 was highly expressed, the involvement of the receptor in ATP-promoted invasion and metastasis of cancer was inevitable (Qiu *et al.*, 2014). What is more, in patients with metastatic prostate cancer, the genetic interactions between single-nucleotide polymorphisms (SNPs) in VEGFR-2 and P2X7 receptors were examined. This analysis indicated that a few SNPs in the VEGFR-2 and P2X7 receptor genotypes may be able to pinpoint a population of prostate cancer patients with a better prognosis of survival (Solini *et al.*, 2015).

P2X7 in bone tumors

High levels of the P2X7 receptor have been detected in many types of tumors of the skeletal system. In osteosarcoma, Ewing's sarcoma, and chondrosarcoma, an increased level of the P2X7 receptor was detected as compared to healthy tissues (Gartland *et al.*, 2001; Alqallaf *et al.*, 2009; Liu & Chen, 2010). Cell lines derived from the backbone are characterized by a wide variety of P2X7 receptor isoforms. The cells of the SaOs-2 lineage express a functional P2X7 receptor capable of forming a pore in the plasma membrane and stimulation of these cells by ATP or BzATP causes increased cell death (Gartland *et al.*, 2001). On the other hand, in the HOS line, administration of extracellular ATP to the culture medium did not cause cell death but increased proliferation (Liu & Chen, 2010). Additionally, when it comes to the properties of the P2X7B split variant in human cancer cell lines, its expression decreased adherence and increased invasion and migration of cells, exhibiting a metastatic phenotype (Tattersall *et al.*, 2021).

Table 2. Summary of the P2X7 receptor presence and activity in the cancer biology.

Type of cancer	Expression of P2X7 receptor (cancer/healthy tissues)	P2X7 variants	Variants capable of forming cell pores	Effect/detection of high level of P2X7 receptor
Prostate cancer	High	No data	No data	Positive correlation with a high grade of tumor aggressiveness and invasiveness
Bone tumors	High	Wide variety of isoforms, mainly P2X7 B	Depends on the cell line (SaOs-2 cell line – detected, HOS cell line – not detected)	Detection in osteosarcoma, Ewing's sarcoma and chondrosarcoma
Skin cancers	High	No data	Detected (but not leading to cell death)	Increased synthesis in normal keratinocyte cells surrounding the tumor
Pancreatic cancer	High	P2X7 A P2X7 B	Detected	Positive correlation with a high grade of tumor invasiveness
Breast cancer	Low/ high (depends on the data)	No data	Depends on the cell line (HT-29 and Colo-205 – not detected, HCT8, Caco-2, MCA38 – detected	Positive correlation with a high grade of tumor migration and invasiveness
Gastrointestinal cancers	High	No data	No data	Positive correlation with a high grade of tumor malignancy
Lung cancers	High	P2X7 B	No data	Detection in non-small-cell lung carcinoma; highest expression in metastatic cancer cells
Blood cancers	High	P2X7 B	Both detected	Detection in childhood leukemias, acute myeloblastic leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia
Cervical cancer	High	P2XJ trimers with P2X7 A	Not detected	Detection in cervical squamous cell carcinoma
Ovarian cancer	High in both groups	No data	Both detected	Increased intensity of proliferation
Neuroblastoma	High	P2X7 B	Not detected	Negative correlation with the survival time of the patients
Glioblastoma	Low	P2X7 B	Detected	Positive correlation with a high grade of tumor aggressiveness

P2X7 in skin cancers

P2X7 receptor protein immunodetection in biopsy tissue material from patients suffering from skin cancer showed higher average levels of this receptor compared to normal tissues. Increased synthesis of P2X7 receptor protein has also been observed in normal keratinocyte cells surrounding the tumor (Slater *et al.*, 2003; White *et al.*, 2005). Increased levels of the P2X7 receptor protein can be found in both animal and human cell lines. In spontaneous murine B16 melanoma, the level of P2X7 receptor synthesis may influence the survival of tumor cells after radiotherapy. The use of a combination of radiotherapy and P2X7 receptor inhibitors significantly increased the treatment efficiency (Tanamachi *et al.*, 2017). Many studies have shown that the administration of P2X7 receptor inhibitors alone significantly inhibited the development of tumors derived from murine B16 melanoma (Adinolfi *et al.*, 2015; De Marchi *et al.*, 2019; Brenet *et al.*, 2021). Moreover, an increase in tumor-infiltrating T cells that express low levels of CD39 and CD73 is another effect of P2X7 pharmacological inhibition in B16-derived tumors (De Marchi *et al.*, 2019). When it comes to B16 melanoma, the stimulation by extracellular ATP caused the formation of the cellular pore but it did not significantly affect cell death (Tanamachi *et al.*, 2017). Studies of human melanoma cells contained in the NCI-60 matrix have shown that increased expression of mRNA encoding the P2X7 receptor is a characteristic feature common to all melanoma cells (Shankavaram *et al.*, 2009; Reinhold *et al.*, 2012). Moreover, in both the human A375 melanoma line and in the murine B16 line, the major variant of the P2X7 receptor is the one capable of pore formation in the plasma membrane (White *et al.*, 2005).

P2X7 in pancreatic cancer

Increased expression of mRNA encoding the P2X7 receptor has been detected in patients with chronic pancreatitis and pancreatic cancer (Künzli *et al.*, 2007). The P2X7 receptor protein has also been detected in cell

lines derived from pancreatic cancer (Giannuzzo *et al.*, 2015). Experiments using human pancreatic cancer cell lines have demonstrated the diversity of P2X7 receptor isoforms present in them. The tested lines could contain the full isoform – P2X7 A – as well as the shortened isoform – P2X7 B. The P2X7 receptors activated by extracellular nucleotides exerted a proliferative effect on human pancreatic cancer cells. The key molecules involved in P2X7 receptor-induced proliferation and cancer growth were protein kinase C (PKC), phospholiase D (PLD), extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), and c-Jun N-terminal kinase (JNK). However, the suppression of inducible nitric oxide synthase (iNOS) by P2X7 receptors indicated their anti-inflammatory role in pancreatic cancer and recovery (Choi *et al.*, 2018). The involvement of the P2X7 receptor in cancer development is also reported in another study. The receptor promotes the proliferation of pancreatic stellate cells (PSC) which are indirectly involved in the progression of pancreatic ductal adenocarcinoma (PDAC). The release of IL-6 cytokine from PSC in response to the P2X7 receptor causes STAT3 activation in pancreatic cancer cells, indicating the importance of P2X7 in the interaction between PSC and cancer cell (Magni *et al.*, 2021). Cells treated with extracellular ATP had a lowered mitotic index (MI) and increased uptake of propidium iodide, which indicates the opening of the cellular pore leading to cell death (Giannuzzo *et al.*, 2015). The same cells were however characterized by increased mobility and invasiveness. The same research group showed the effect of the P2X7 receptor on the invasiveness of these cells *in vivo* in an orthotopic pancreatic cancer model (Giannuzzo *et al.*, 2016). The administration of the P2X7 receptor inhibitor significantly reduced the tumor volume and the tumor-related fibrotic changes in the pancreas.

P2X7 in breast cancer

The role of the P2X7 receptor activity in breast cancer is much more complex and unclear than in the cases described above. Some researchers indicate a reduced P2X7 synthesis in invasive lobular adenocarcinoma and pancreatic ductal adenocarcinoma compared to normal tissues (Li *et al.*, 2009). Moreover, studies with the use of micro-RNA molecules (miR-150) regulating the synthesis of P2X7 protein have shown that such a reduction in the level of the P2X7 receptor may enhance the development of breast cancer. Cell lines incubated with miR-150 molecules showed increased aggressiveness and invasiveness along with a decrease in P2X7 protein levels. The same results were obtained during *in vivo* experiments, where administration of miR-150 inhibitors increased P2X7 protein synthesis and thus decreased the aggressiveness of tumors in an orthotopic model of breast cancer (Huang *et al.*, 2013). Similarly, in a different study, BzATP, a P2X7 receptor agonist, significantly increased cancer cell migration and invasion in MCF-7 breast cancer, which effect could be blocked by the P2X7 receptor antagonists (Sharma *et al.*, 2021). There are however results, standing in contradiction with the aforementioned. A study by Tan in 2015, showed increased production of the P2X7 protein in cancer cells compared to normal cells. Moreover, experiments using small interfering RNA have shown that inhibition of receptor synthesis may negatively affect the proliferation of MCF-7 cells. In addition, the downregulation of P2X7 initiated apoptosis in this line. Such studies also showed a positive correlation between the level of the P2X7 receptor and the level of the estrogen receptor (Tan *et al.*, 2015). The observed discrepancy in the studies on the P2X7 receptor level in normal and cancer tissues may be related to variants of this protein. The use of an antibody detecting the C-terminus of a protein will not detect truncated variants of the receptor. Studies with an antibody that detects the extracellular fragment showed an increased level of the P2X7 receptor compared to normal tissues (Slater *et al.*, 2004b). The above examples show how complex the receptor systems in cancer cells can be and how much the research should use methods detecting possible alternative variants of the assembly of transcripts encoding the P2X7 receptor.

The changed level of P2X7 receptor production does not have to be a result of cancer cells expression profile. It may also be associated with adverse environmental conditions in the tumor. The conditions of the lowered oxygen level increased the mRNA and protein of the P2X7 receptor synthesis (Tafani *et al.*, 2010). Additionally, breast cancer patient's response to treatment was correlated with purinergic signaling of the P2X7 receptor. In contrast to chemoresistant people, non-chemoresistant patients showed a higher level of P2X7 receptor expression CD8+ T cells. Patients with chemoresistance exhibited altered P2X7 receptor activity, which was demonstrated by a constant number of CD8+ T cells activation marker and a decreased level of IFN-γ production in the presence of ATP (Ruiz-Rodríguez *et al.*, 2020). What is more, the application of P2X7 ability to promote cancer cell death in high ATP conditions was described in Draganov study in which the anti-cancer properties of Ivermectin were mediated by differential ATP/P2X7-dependent cytotoxicity (Draganov *et al.*, 2021).

P2X7 in gastrointestinal cancers

In normal, healthy stomach tissue, the synthesis level of the P2X7 receptor remains at a low level. With the appearance of cancer changes, the level of this protein increases. Moreover, the level of P2X7 synthesis is correlated with the stage of tumor development. The more malignant the tumor is and a later stage of development progress, the greater the level of the P2X7 receptor. The above results suggest that the P2X7 receptor may be also a prognostic factor for the malignancy of gastric cancer and the estimated survival time of the patient (Calik *et al.*, 2020).

A more complicated expression pattern is found in colorectal cancer. Analysis of postoperative fragments from 97 patients suffering from colorectal cancer showed a wide range of levels of P2X7 receptor synthesis compared to normal tissues. Moreover, patients with high levels of P2X7 receptor lived significantly shorter compared to patients with low levels of P2X7 receptor synthesis (Zhang *et al.*, 2019). Several variants of the P2X7 receptor have been detected in colorectal cancer. There were functional variants that formed the cell pore as well as shorter variants that lacked this function (Barden, 2014). A similar pattern of P2X7 receptor expression occurs in cell lines. HT-29 and Colo-205 lines are characterized by the presence of shorter P2X7 receptor isoforms devoid of the ability to cell pore formation (Barden *et al.*, 2003), while the HCT8, Caco-2, and MCA38 lines are characterized by the presence of the complete form of the P2X7 receptor which forms the functional cell pore (Coutinho-Silva *et al.*, 2005; Künzli *et al.*, 2011; Bian *et al.*, 2013).

In colon cancer, patients with omental and peritoneal carcinomatosis had greater levels of P2X7 receptor with

NLRP3 inflammasome in their adipocytes. The complex showed a correlation with the levels of circulating white blood cells (WBC) and the chemotactic factor MCP-1 implicated in tissue infiltration of monocyte and macrophages. These data indicate the involvement of the P2X7 receptor together with the NLRP3 inflammasome in modulating chemotaxis and spread of the metastasis in colon cancer (Solini *et al.*, 2021).

P2X7 in lung cancer

Transcripts encoding the P2X7 receptor were found in 26 patients suffering from non-small-cell lung carcinoma. Interestingly, in the same studies, no P2X7 transcripts were detected in patients with a chronic obstructive pulmonary disease which may indicate an association of this receptor presence with the appearance of cancer in the lungs. Additionally, the highest expression of mRNA encoding P2X7 was found in metastatic cancer cells (Schmid *et al.*, 2015). The P2X7 receptor is also present in the cancer cell lines of the non-small-cell lung carcinoma, A549, PC-9, and H-292 (Takai *et al.*, 2012, 2014) and is completely absent from normal bronchial epithelial cells, Beas-2B (Takai *et al.*, 2014). In other studies, both tumor and immune cells of lung adenocarcinoma were shown to express the P2X7 receptor, but only immune cells did so with a functioning receptor. The tumors with a high expression of the P2X7B split variant were less infiltrated with B and T cells and more infiltrated with myeloid cells. P2RX7B differential expression positively influenced tumor development by controlling the regulation of the quality of immune cell infiltration (Benzaquen *et al.*, 2020). On the other hand, a small-molecule P2X7 receptor activator improves immune surveillance and stimulates anti-tumor immune responses by enabling the effector functions of adaptive immune T cells. These actions increase the effectiveness of αPD-1, an immune checkpoint inhibitor, in the treatment of non-small cell lung cancer (Douguet *et al.*, 2021). In H-292 cells, the reduction of P2X7 receptor levels decreased actin cytoskeleton remodeling and migration evoked by transforming growth factor beta (TGF-β) (Takai *et al.*, 2014).

P2X7 in blood cancers

P2X7 is also widely distributed in various types of leukemia but there is no uniform pattern in the function of this receptor. In mouse acute erythroleukemia cells (MEL), the P2X7 receptor may form a functional cell membrane pore which leads to cell death (Constantinescu *et al.*, 2010). P2X7 receptor levels are also higher in childhood leukemias (Chong *et al.*, 2010) and it has also been detected in patients with acute myeloblastic leukemia and acute lymphoblastic leukemia (Zhang *et al.*, 2004). Moreover, in the cells of patients with acute myeloblastic leukemia (AML), the increased level of the P2X7 receptor correlated with the shorter survival time of the patients. When it comes to acute myeloid leukemia, it was determined that P2X7A and P2X7B receptor isoforms were overexpressed, demonstrating a favorable correlation between the progression of the disease and both receptor variations. In relapsing patients, the level of P2X7A and P2X7B receptors was downmodulated and upmodulated, respectively, whereas in remitting AML patients both P2X7A and P2X7B receptors expression was decreased. Daunorubicin (DNR), which is one of the primary chemotherapeutic agents for AML, changed the level of P2X7A and P2X7B receptors in AML blasts to the same as in the relapsing patients. In the presence of high ATP levels caused by DNR administration, the P2X7A receptor produced by AML blasts promoted the opening of a large nonselective pore, which allowed the intracellular absorption of anticancer drugs that caused cell death. In contrast, the increase in ATP promoted AML relapse by enabling the growth of P2X7B receptor-expressing blasts, which were unable to form the cytotoxic pore but were still able to activate the channel function of the receptor which protected cells from death caused by chemotherapy (Pegoraro *et al.*, 2020). In a different study, AML development was effectively postponed both *in vitro* and *in vivo* by blocking ATP/P2X7 signaling with the particular antagonist. The P2X7 receptor was enhancing leukemogenesis by promoting the self-renewal and homing abilities of leukemia-initiating cells (He *et al.*, 2021). Additionally, the P2X7 receptor accelerated the progression of AML in patients with mixed lineage leukemia (MLL) gene correlated with poor prognostics. Through the activation of the leukemia transcription factor Pbx3, the receptor promoted the proliferation of leukemia stem cells which resulted in the development of AML (Feng *et al.*, 2021). The P2X7 receptor has also been detected in chronic lymphocytic leukemia cells in which the level of the P2X7 receptor has correlated with the severity of the disease (Adinolfi *et al.*, 2002). Other studies related to chronic lymphocytic leukemia have shown the presence of two variants of the P2X7 receptor in these cells. In addition to the complete cell pore-forming receptor, these cells have also produced a truncated form of the P2X7 receptor that did not form the cell pore (Gu *et al.*, 2000).

P2X7 in cervical cancer

In cervical squamous cell carcinoma (CSCC), an immunodetection study of P2X7 receptor variants that did not form the cell pore showed higher levels of this protein compared to healthy tissues (Barden, 2014). The higher risk of HPV-16 positive CSCC development was associated with inherited dysfunction in the P2X7 receptor caused by single nucleotide polymorphisms (Yang *et al.*, 2016). Moreover, other studies showed a decreased level of the full-length variant of the P2X7 receptor, where the level of reduction correlated with the degree of tumor development (Li *et al.*, 2007). Similarly, in the two separate human cervical cancer cell lines, the P2X7 receptor would become engaged in the anticancer activity of atractylenolide-I (Han *et al.*, 2022). There are also reports indicating the coexistence of different P2X7 receptor isoforms in cervical cancer cells, where an alternative P2XJ receptor splicing variant may form non-functional trimers with the P2X7A variant (Feng *et al.*, 2006).

P2X7 in ovarian cancer

The immunodetection of the P2X7 receptor protein showed a high level of this receptor in both: patients with ovarian cancer and in healthy tissue donors. The level of the receptor in healthy and pathological tissues did not differ significantly. Interestingly, in the same studies, cell lines from patients with SKOV-3 and CAOV-3 ovarian cancer showed an increased level of the P2X7 receptor and an increased intensity of proliferation after stimulation by ATP and BzATP (Vázquez-Cuevas *et al.*, 2014). Other studies have shown that in the biopsy material from patients with ovarian cancer, the P2X7 receptor variant that forms the cell pore and shorter pore non-forming variants occur simultaneously (Gilbert *et al.*, 2019).

P2X7 in neuroblastoma

Studies using samples from patients suffering from neuroblastoma showed a high level of P2X7 protein synthesis regardless of the stage of the disease. Neuroblastoma commercial cell lines are also characterized by the presence of the P2X7 protein (Raffaghello *et al.*, 2006). Moreover, the stimulation of neuroblastoma cells by extracellular ATP did not cause cell death but the intensification of cell divisions and increased secretion of substance P. This may suggest that there is a shorter variant of the P2X7 receptor in neuroblastoma cells, which does not cause cell death by pore formation in the membrane (Raffaghello *et al.*, 2006). Other studies have shown robust expression of mRNA encoding the P2X7 receptor in samples from 131 patients suffering from neuroblastoma (Amoroso *et al.*, 2015). It has also been shown that there is a negative correlation between the high expression of the P2X7 gene and the survival time of the patients. Studies using ACN cells showed that lowering the P2X7 receptor level with small interfering mRNA decreased AKT kinase phosphorylation (*in vivo* and *in vitro*) and the level of vascularization of cancer tumors *in vivo* (Amoroso *et al.*, 2015). Moreover, it was indicated that the cooperation between kinin and purinergic signaling networks is crucial for neuroblastoma to spread and metastasize to the bone marrow. The treatment with bradykinin, a pro-metastatic factor, increased the expression levels of the P2X7B isoform in comparison to the P2X7A receptor. As a result, the compound increased tumor proliferation, which P2X7 receptor antagonists greatly reduced. However, bradykinin did not increase cell death or P2X7A receptor-related pore activity, favoring the development of neuroblastoma (Ulrich *et al.*, 2018). Additionally, alternative forms of non-functional P2X7 receptor (nfP2X7) were detected in neuroblastoma. The study indicated that the nfP2X7 receptors were necessary for the survival of the tumor cells. The expression of those receptors was stimulated by high ATP concentrations, which characterize the tumor microenvironment. High concentrations of ATP promoted a transition from P2X7 to nfP2X7. This switch allowed tumor cells to take advantage of nfP2X7 ability to promote cell survival and proliferation without suffering the consequences of large pore-mediated cell death, like in the case of P2X7 receptors (Gilbert *et al.*, 2019).

P2X7 in glioblastoma

Glioblastomas are the most common and malignant primary brain tumors in adults. These tumors are also characterized by the highest degree of malignancy, frequent relapses, rapid growth, and destruction of tissues adjacent to the tumor. The life expectancy of a person diagnosed with glioblastoma ranges from several months to two years after diagnosis (Kleihues *et al.*, 2002; Wen & Kesari, 2008). The high mortality is not only due to the malignancy of the tumor but also to difficult diagnostics and treatment. As tumors develop behind the blood-brain barrier, chemotherapy is limited to a few orally administered chemotherapeutic agents such as Temozolomide (Bush *et al.*, 2017), able to cross this barrier. Radiation therapy is not always efficient either. This is due to the different resistance to ionizing radiation in patients and the highly hypoxic nature of glioblastoma which hinders the effects of radiotherapy (Amberger-Murphy, 2009). The most commonly used treatment strategy that ensures the longest disease-free time and improves the patient's life is the surgical removal of the tumor along with the surrounding tissues (Li *et al.*, 2016b). However, due to the cancer cells' tendency to infiltrate surrounding tissue, it is almost impossible to remove the tumor completely and the residual tumor remnants are left behind to cause the disease to relapse soon.

The P2X7 receptor is, along with other nucleotide receptors, widely distributed in all cells that make up the brain tissue (Collo *et al.*, 1997; Duan & Neary, 2006; Yu *et al.*, 2008; Jimenez-Mateos *et al.*, 2019). Not surprisingly, this receptor is also observed in many pathological conditions in the brain, including glioblastoma. The P2X7 receptor is present in almost all cell models of glioblastoma but its role in the development of this disease is unclear. Analysis of the protein level and expression of the gene encoding P2X7 showed a decreased level of the P2X7 receptor in glioma as compared to the healthy brain. The authors of the study explained this phenomenon by the strong methylation of the gene encoding P2X7 (Liu *et al.*, 2017).

In studies using the murine glioblastoma line GL261, this receptor increases susceptibility to cell death and reduces resistance to radiotherapy. Decreasing the P2X7 receptor level using small interfering RNA did not reduce tumor size in the *in vivo* model. However, tumors with decreased levels of this receptor were significantly less responsive to radiation therapy (Gehring *et al.*, 2015). Tamajusuku showed similar results indicating the positive role of the P2X7 receptor in stimulating cell death *in vitro*. Mouse GL261 glioma cells, stimulated by extracellular ATP, died as a result of necrosis (Tamajusuku *et al.*, 2010). Moreover, in this cell line, the P2X7 receptor is probably functional and its activation results in a calcium signal and a cell pore formation, leading to cell death. Studies by Strong's group showed an increase in calcium signal when stimulated by extracellular ATP. The stimulation of the receptor in a medium with a reduced content of calcium ions significantly reduced the number of responding cells. Moreover, the addition of the P2X7 receptor permanent inhibitor – oxATP – also decreased the number of responding cells (Strong *et al.*, 2018). In conclusion, in the murine model of GL261 glioma, the P2X7 receptor functions as a receptor regulating cell death, and its stimulation by extracellular ATP may enhance the anti-tumor effect in therapy.

Studies of the function of the P2X7 receptor that occurs in rats' glioblastoma are much more confusing. Studies using extracellularly administered apyrase, an enzyme that hydrolyzes ATP, showed that applying it to the area of the implanted C6 glioma tumor reduced its volume. Moreover, the effects of tumor inhibition were similar to those obtained in the group of animals treated with temozolomide (Morrone *et al.*, 2006). However, the functionality of the P2X7 receptor in C6 glioma cells *in vitro* remains unclear. Some researchers believe that the calcium signal appearing after the administration of ATP or BzATP comes from the stimulation of the P2X7 receptor, and that stimulation of this receptor increases the aggressiveness of C6 glioma cells and the expression of this receptor itself (Wei *et al.*, 2008). Also, other studies showed the appearance of a calcium signal after stimulation by BzATP and increased intensity of proliferation with no signs of cell death (Matyśniak *et al.*, 2020). On the other hand, studies on the calcium signal in C6 glioma also showed the possibility of P2Y2 receptor stimulation by BzATP and the lack of cellular pore formation after P2X7 receptor stimulation (Suplat-Wypych *et al.*, 2010). Other research groups indicate the TRPM7 ion channel (transient receptor potential cation channel, subfamily M, member 7) as a source of calcium signal after BzATP administration in C6 glioma cells (Nörenberg *et*

al., 2016). It should be noted, however, that so far, no studies using small interfering RNA or other genetic engineering methods have been performed that could help test P2X7 activity in these cells *in vitro*.

In vivo studies with rat C6 glioblastoma show a clear and significant role of the P2X7 receptor in regulating tumor aggressiveness. Administration of an inhibitor of this receptor (BBG) to rats with C6 glioma led to a significant reduction in the size of tumors and the amount of infiltrating microglia in the tumor mass (Ryu *et al.*, 2011). Moreover, the influence of the P2X7 receptor on the secretion of macrophage inflammatory protein (MIP-1α), which may promote microglia recruitment and support the aggressiveness of C6 glioma has also been demonstrated (Fang *et al.*, 2011). However, there is also a report in the literature that the P2X7 receptor has an inhibitory effect on the development of C6 glioma. Studies by Fang *et al.* have shown that inhibition of the P2X7 receptor increases tumor size and increases expression of the P2Y2 receptor, hypoxia-inducible factor 1 (HIF-1α), and vascular endothelial growth factor (VEGF) (Fang *et al.*, 2013).

The expression pattern and functional properties of the P2X7 receptor in human glioma cells are similarly complex. The P2X7 receptor is present in almost all studied lines of human glioblastoma (Ji *et al.*, 2018; Bergamin *et al.*, 2019; Matyśniak *et al.*, 2020) and patient tissues (Ziberi *et al.*, 2019). However, the functionality of this receptor differs depending on the cell line. All aforementioned research groups working on this issue clearly indicate the lack of P2X7-induced cell death in human glioblastoma cultures. However, this is where the consistency of the results ends. Ji *et al.* showed that stimulation of the P2X7 receptor by the agonist BzATP positively influenced the proliferation of human glioma cells. However, studies of other groups do not confirm these reports (Bergamin *et al.*, 2019; Matyśniak *et al.*, 2020). Inhibition of P2X7 receptor activity influenced tumor size in the *in vivo* model of human U-138 glioma (Bergamin *et al.*, 2019). Also, in the human U-251 glioma model, blocking P2X7 receptor activity inhibited cell proliferation and the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF). Moreover, inhibition of cell growth following administration of a P2X7 inhibitor had the same effect in inhibiting proliferation as treatment of cells with temozolomide (Kan *et al.*, 2020; Drill *et al.*, 2020).

Extracellular ATP may also influence the development of spheroids *in vitro* as well as the share of glioblastoma stem cells in all tumor cells population. ATP administered to the culture medium decreased the level of stem cell markers and decreased the size of the spheroids of the human glioma lineage (Ledur *et al.*, 2012). However, studies using glioblastoma stem cells isolated from patients have shown the stimulating role of the P2X7 receptor on the development of stem cells and the presence of epithelial-mesenchymal transition (EMT) markers which contribute to increased cell migration (Ziberi *et al.*, 2019).

There are also reports of an inhibitory effect of glioblastoma P2X7 receptor on radiation-sensitive human M059J glioblastoma. Cell irradiation increased P2X7 receptor synthesis in these cells and increased the number of cells entering the cell death pathway (Gehring *et al.*, 2012). Similarly, after cell irradiation, a shift in the P2X7 isoform expression was detected – the P2X7A and P2X7B isoforms were down- and upregulated, respectively. These emerged clones resistant to the radiation were responsible for tumor recurrence. The combination of radiotherapy with P2X7R-targeting drugs was a more effective treatment than radiation alone – treatment with P2X7 receptors antagonists during the recovery phase increased irradiation-dependent cytotoxicity and cell death in GB40 and GB48 cells (Zanoni *et al.*, 2022).

Summarizing, a heterogeneous picture emerges from the research on the role of the P2X7 receptor. This receptor appears to be involved in many cellular functions but to fully understand its role in glioblastoma, more in-depth research is needed to reveal molecular interactions and mechanisms.

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