

THE POTENTIAL OF USING THE BIOLUMINESCENT SYSTEM OF *CHAETOPTERUS VARIOPEDATUS* TO STUDY FERROPTOSIS IN LIVING ORGANISMS

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Ferroptosis is a form of programmed cell death associated with iron-dependent lipid peroxidation. Novel ferroptosis inducers and suppressors could be instrumental in developing drugs against neurodegenerative disorders and cancer. Prior to embarking on a search for ferroptosis inducers/suppressors, this form of cell death must be studied in living cells and laboratory animals. In addition to two cofactors, luciferase (or photoprotein) of the parchment tubeworm *Chaetopterus varioopedatus* requires the presence of iron ions and hydrogen peroxide or organic hydroperoxides to exert its activity. Therefore, the bioluminescence system of the parchment tubeworm can be used to study ferroptosis in living organisms.

Keywords: ferroptosis, lipid peroxidation, bioluminescence, luciferase, luciferin, bioimaging, biomedical research, parchment worm, *Chaetopterus*

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ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ БИОЛЮМИНЕСЦЕНТНОЙ СИСТЕМЫ *CHAETOPTERUS VARIOPEDATUS* ДЛЯ МОНИТОРИНГА ФЕРРОПТОЗА В ЖИВЫХ ОРГАНИЗМАХ

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Ферроптоз — особый тип программируемой гибели клеток, связанный с интенсивным окислением липидов под действием свободных ионов двухвалентного железа. Новые индукторы и супрессоры ферроптоза могут стать основой для создания препаратов для лечения нейродегенеративных и онкологических заболеваний. Для поиска таких индукторов (супрессоров) ферроптоза необходимо детектировать этот процесс в живых клетках или у лабораторных животных. Люцифераза (фотопротейн) морского дракона *Chaetopterus varioopedatus* для своей активности требует помимо двух кофакторов наличия ионов железа и пероксида водорода (либо органических гидропероксидов). Соответственно биоломинесцентная система морского дракона может быть использована для мониторинга ферроптоза в живых организмах.

Ключевые слова: ферроптоз, окисление липидов, биоломинесценция, люцифераза, люциферин, биоимиджинг, биомедицина, морской дракон, *Chaetopterus*

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Ferroptosis is a new form of programmed cell death

Programmed cell death is cell death coordinated by specific intracellular mechanisms. It has been described in higher animals, invertebrates, plants, bacteria, and fungi. Programmed cell death plays an immense role in normal organismal development and functioning [1]. It is essential for embryonic development and morphogenesis, tissue remodeling, immune response, elimination of malignant and infected cells. Excessive or insufficient programmed cell death leads to neurodegenerative, autoimmune, oncological and other diseases. The importance of studying programmed cell death was emphasized by two Nobel prizes in physiology and medicine in 2002 and 2016. The first form of programmed cell death to be discovered was apoptosis [2]. Today, at least 10 different nonapoptotic pathways of programmed cell death are known, including autophagy-dependent cell death,

necroptosis, ferroptosis, pyroptosis, parthanatos, entosis, NETosis, and some others [1]. Despite extensive research efforts, our knowledge about these processes is still limited.

Ferroptosis is a form of programmed cell death that might hold promise for practical applications. In 2004, it was discovered that a hitherto novel heterocyclic compound erastin caused cell death via a previously unknown nonapoptotic pathway [3]. Erastin directly interacts with mitochondrial voltage-dependent anion channels (VDAC) and its action is inhibited by antioxidants, such as α -tocopherol, butylated hydroxytoluene and β -carotene [4]. It is reported that erastin-induced cell death can be prevented by ferrous iron chelators [5]. In 2012, Dickson et al. proposed the term *ferroptosis* to refer to a new form of cell death associated with intense iron-dependent lipid peroxidation. Genetically, biochemically and morphologically, ferroptosis is distinct from apoptosis, necrosis and autophagy-dependent cell death [1, 6]. Unlike apoptosis, ferroptosis is not

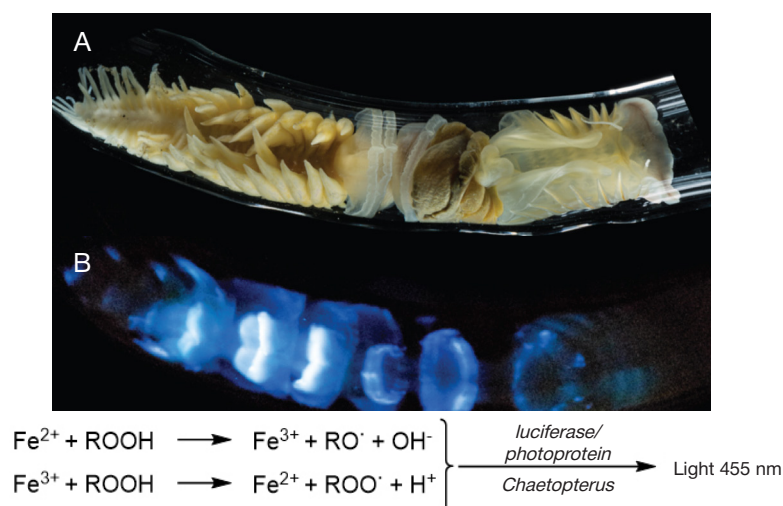


Fig. Bioluminescence of *Chaetopterus variopedatus*; top: a parchment tubeworm outside of its tube in the light (A) and in the dark (B) (photos provided by Alvaro E. Migotto and Anderson G. Oliveira); bottom: a possible mechanism of bioluminescence (after Mirza JD et al., 2020)

accompanied by cell nucleus transformation and chromatin condensation and is characterized by mitochondrial shrinkage, increased mitochondrial membrane density and vanishing of mitochondrial cristae [5, 6]. The loss of glutathione peroxidase 4 (GPX4) activity leads to iron-dependent lipid peroxidation and the accumulation of lipid hydroperoxides in the cell, resulting in ferroptotic cell death [5, 7]. Ferrous ions accumulation and lipid hydroperoxide generation, which cause cell death, are seen in some neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, Friedreich's ataxia, and other pathologies, such as amyotrophic lateral sclerosis, periventricular leukomalacia, ischemic stroke, traumatic brain injury and acute kidney injury. Development and progression of these disorders are thought to be associated with the activation of the ferroptotic cascade [8]. At the same time, ferroptosis appears to be a promising therapeutic strategy for the treatment of apoptosis-resistant malignant tumors, since cancer cells contain increased amounts of ferrous iron. For example, sorafenib, a drug effective against renal cancer, has been shown to induce ferroptosis [9]. Thus, research into the mechanisms underlying ferroptosis and the search for new ferroptosis inducers/suppressors may facilitate the development of novel drugs against neurodegenerative diseases and cancer. However, prior to a large-scale screening of candidate ferroptosis inducers and suppressors, an effective monitoring system is needed to track this process in living cells and laboratory animals.

The promise of the *Chaetopterus variopedatus* bioluminescent system

Modern biotechnology and diagnostics actively exploit bioluminescence-based methods characterized by high sensitivity and low background signal. Bioluminescence systems are employed for analyte detection, immunological testing, gene expression analysis, drug screening, bioimaging of living organisms, visualization of intracellular structures and

processes, cancer studies and infectious diseases research, and environmental monitoring [10]. Bioluminescence systems are common in nature, but few of them have been well characterized and adapted for practical use. The deciphering of yet understudied bioluminescence systems is instrumental in creating new analytical techniques [11]. One such system is the underinvestigated and unusual bioluminescence system of the parchment tubeworm *Chaetopterus variopedatus* (see Figure). These benthic filter-feeding organisms inhabit the tube-shaped "homes". When pulled out of the tubes, the worms emit bright blue light and secrete luminescent mucus. The biological role and molecular underpinnings of this mechanism are still not fully clear [12]. According to earlier reports, the activity of *Chaetopterus variopedatus* photoprotein is dependent on the presence of two cofactors, ferrous iron and hydrogen peroxide or organic hydroperoxides [13]. Later reports demonstrated that the iron-dependent bioluminescent system of the parchment tubeworm functions like the classic "luciferin-luciferase" system [14].

As ferroptosis is characterized by simultaneous accumulation of ferrous iron and generation of reactive oxygen species, the ferroptotic cells expressing *Chaetopterus variopedatus* luciferase would develop a luminescent signal.

Conclusion

Programmed cell death plays an immense role in normal organismal development and maintenance. Dysregulation of programmed cell death leads to neurodegenerative and autoimmune disorders, cancer, etc. Ferroptosis is a nonapoptotic form of programmed cell death associated with intense iron-dependent lipid peroxidation. Research into the mechanisms underlying ferroptosis and the search for new ferroptosis inducers and suppressors has practical implications. The bioluminescent system of the parchment tubeworm is a promising tool for studying ferroptosis in living laboratory organisms.

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