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A Call for Innovation in Drug Screening: Integrating Genetically Encoded Biosensors for Deeper Insights

İlaç Taramasında Yenilik Çağrısı: Daha Derin Bilgiler İçin Genetik Olarak Kodlanmış Biyosensörlerin Entegrasyonu

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Dear Editor,

We are writing to express our views on the urgent need for more informative and efficient methods in drug screening and testing within preclinical and translational medicine. The current state of the art primarily involves 2,5-diphenyl-2H-tetrazolium bromide (MTT) or similar cell viability assays, which provide limited information, predominantly focusing on cell toxicity. These methods are poor in providing insights into specific cellular mechanisms, long-term effects, or the drug's mode of action, thereby failing to deliver the comprehensive analysis required in modern drug development (1).

In vivo experiments, specifically in the context of drug development, are increasingly being abandoned in both the EU and the USA due to ethical concerns and regulatory changes. As a result, the reliance on in vitro systems and the development of innovative tools for drug screening have become more crucial than ever. Among these, genetically encoded biosensors for high-content drug screening represent a highly promising yet underutilized approach in the field.

Genetically encoded biosensors are advanced tools designed to provide real-time insights into cellular processes. Engineered from natural or synthetic genetic elements, these biosensors are optimized for high-throughput applications, allowing detailed monitoring of cellular responses. They track various biological signals or molecules, offering precise data on drug mechanisms and interactions at the molecular level (2). Integrating these biosensors into drug screening protocols can significantly enhance the efficiency and effectiveness of drug development processes by providing critical, functional insights into how drugs impact cellular functions.

Some of our previous studies have highlighted the significant value and effectiveness of genetically encoded biosensors in this field. These biosensors have proven particularly valuable in studying nitric oxide (NO), a crucial signaling molecule in the cardiovascular system (3). NO is essential for regulating vascular tone, blood pressure, and overall cardiovascular health by activating soluble guanylate cyclase (sGC), leading to vasodilation and maintaining endothelial function and vascular homeostasis. In the context of cardiovascular therapeutics, nitroglycerin (GTN) is a well-established antianginal drug that exerts its effects through NO release. The bioactivation of GTN by aldehyde dehydrogenase-2 (ALDH2) converts it to NO, which then activates sGC and induces vasodilation. Despite GTN's clinical success, understanding the precise mechanisms of its NO release and its impact on NO signaling pathways has been challenging due to limitations in traditional measurement techniques. Our research has demonstrated how advancements in genetically encoded biosensors can overcome these challenges. These innovative tools provide important insights into the real-time dynamics and spatial distribution of NO within cells, enabling a more precise visualization of NO production and its effects on cellular signaling. Our study, employing NO biosensors, has notably advanced the understanding of NO's role in cardiovascular pharmacology.

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It has addressed longstanding questions about NO signaling in response to GTN, highlighting the intricate interplay between drug metabolism and NO production in the vascular system (4).

In another study, we assessed the efficacy of two approved iron supplement drugs – Venofer and Ferinject- using our genetically encoded biosensor approach. This method effectively detects available iron (II) in cultured cells, demonstrating that genetically encoded biosensors are valuable tools for drug screening and testing at the (sub)cellular level (5).

Another crucial point we address is the critical importance of physiological oxygen levels in drug testing. We discovered that cells cultured under physiological normoxia (5 kPa O_2) require notably lower iron supplementation to activate metalloproteins compared to cells grown in standard room air (18 kPa O_2) (6). Additionally, we used genetically encoded biosensors to investigate the complex interaction between hydrogen peroxide (H_2O_2) and nitric oxide (NO) in endothelial cells under varying oxygen conditions. Traditionally, simultaneous measurement of these molecules has been technically challenging, leading to inconsistent results. By employing advanced biosensors -HyPer7 for H_2O_2 and geNOps for NO- for concurrent imaging in single cells, we found that, under ambient oxygen conditions, H_2O_2 did not significantly impact NO production. However, under physiological normoxia, we observed distinct oxidative stress and nuanced NO responses to H_2O_2 (7). These results underscore the necessity of incorporating physiological conditions into cellular studies to obtain accurate, meaningful insights, thereby enhancing the reliability of drug testing protocols.

We believe that integrating genetically encoded biosensors into standard drug screening protocols could significantly enhance the development of safer and more effective therapeutics. Their ability to provide detailed functional insights at the cellular level addresses a critical gap left by traditional drug testing methods.

Thank you for considering our perspectives on this matter. We look forward to further discussions and collaborations to advance the field of drug screening and testing.

Sincerely.

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