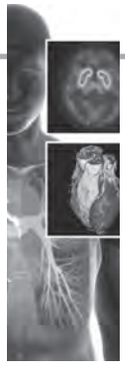

Methods and benefits of imaging the temperature distribution inside living cells

"...*intracellular thermometry, a new method of cell monitoring, could provide information regarding the status of the living cell, and ultimately, information regarding the status of the entire body.*"

KEYWORDS: drug discovery ■ fluorescence lifetime imaging microscopy ■ fluorescent thermometer ■ human disease ■ metabolic activity ■ temperature imaging

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Temperature is the most important physical property for living organisms. This importance is easily understood, as we utilize body temperature as a simple indicator of our health status. The health status of all organisms is influenced by countless chemical reactions occurring within their cells. All such intracellular chemical reactions are accompanied by either heat release (exothermic) or heat absorption (endothermic), resulting in spatial temperature variation within the living cell. This local temperature variation could affect certain cellular functions, such as gene expression, protein stabilization, enzyme–ligand interactions and enzyme activity [1]. Thus, intracellular thermometry, a new method of cell monitoring, could provide information regarding the status of the living cell, and ultimately, information regarding the status of the entire body.

For an accurate and meaningful measurement of intracellular thermometry, a thermometer with several specific capabilities is required. It should be small in size, exhibit a high temperature resolution (i.e., high sensitivity) and demonstrate high spatial resolution, low toxicity and functional independence from other intracellular environmental factors, such as pH and ionic strength. For sensors with a high spatial resolution, a fluorescent probe is an obvious choice and recent developments in novel fluorescent molecular thermometers have opened the door to intracellular thermometry methods [2,3]. In particular, the fluorescent polymeric thermometer (FPT), which we developed, has successfully revealed the intracellular temperature distribution in combination with fluorescence lifetime imaging microscopy (FLIM) [4]. In this editorial, we briefly summarize the recent advances in the development of fluorescent molecular thermometry, with a particular focus on the FPT-FLIM developed by our group. To date, our FPT-FLIM is the only method that is appropriate for quantitatively analyzing the intracellular temperature distribution. In addition, we discuss the meaning of intracellular local temperature differences and their relationship with cellular function based on our results. Finally, the potential medical applications of intracellular temperature imaging using FPT-FLIM are discussed.

Recent advances in fluorescent molecular thermometers

Within the past 2 years, several research groups have reported the use of fluorescent thermometers for monitoring intracellular temperature dynamics, including our FPT method. Each fluorescent thermometer has been designed based on the unique characteristics of a specific molecule, for example, streptavidin-coated quantum dots have been employed by Yang *et al.* [5]; CdSe-CdS quantum dot/quantum rod have been used by Cohen *et al.* [6]; green fluorescent protein has been employed by Quidant *et al.* [7]; and the L-DNA molecular beacon has been used by Yang *et al.* [8]. Quantum dot-based thermometers are considered robust and streptavidin-coated quantum dots have been successfully used to reveal the inhomogeneity of heat generation inside of a living NIH/3T3 cell [5]. Biorelevant macromolecules, such as proteins and nucleic acids, have an advantage owing to their biocompatibility (i.e., low toxicity). However, to date, the results obtained using these fluorescent thermometers have been qualitative, and intracellular temperature mapping has not been achieved.

Future Medicine part of fsg

Recently, our thermoresponsive polyacrylamide-based FPT was demonstrated to have an incomparable sensitivity for the intracellular temperature distribution within mammalian cells in a steady state. This superior sensitivity originates from the functional role assigned to these structures – that is, a thermoresponsive moiety and a signaling fluorescent moiety [9]. A hydrophilic ionic moiety was also incorporated into the FPT to prevent undesired interactions with biorelevant macromolecules, as well as self-aggregation under conditions of intracellular high ionic strength. This FPT displays an increased fluorescence intensity and elongated fluorescence lifetime with increasing temperature. In particular, the fluorescence lifetime of the FPT is independent of the experimental fluctuations, such as the concentration of the FPT, intracellular pH and ionic strength, as well as excitation power. It shows a remarkable sensitivity to the temperature variation (4.6 ns at 28°C and 7.6 ns at 40°C). Thus, we adopted time-correlated single photon counting-based FLIM [10] to analyze the intracellular temperature distribution of COS7 cells. After the FPT was microinjected into the cells, imaging of the intracellular temperature distribution could be performed at a high temperature resolution (0.18–0.58°C) and a high spatial resolution (~200 nm) using time-correlated single photon counting FLIM [4].

Intracellular temperature distribution revealed using FPT-FLIM

Using FPT-FLIM, intracellular temperature imaging revealed organelle- and cell function-dependent heat production inside of the living cell. Initially, we observed that the temperature within the nucleus was significantly higher than in the cytoplasm in the majority of cells, with an average temperature differential of 0.96°C. Further analysis using synchronized cell cultures revealed that this temperature difference between the nucleus and the cytoplasm changed depending on the cell cycle. The temperature difference of the cells in G1 phase was larger (0.7°C on average), whereas there was a smaller temperature difference in S/G2 cells (-0.03°C). This decrease in the temperature difference in S/G2 cells was caused by an increase in the cytoplasmic temperature rather than a decrease in the nuclear temperature.

Significant local heat production was also observed as a single hot spot in the perinuclear region, which was identified as the centrosome using immunostaining analysis with an anti-γ-tubulin antibody. The average temperature difference between the centrosome area and other areas in the cytoplasm was 0.75°C.

> *"[The combination of a fluorescent polymeric thermometer and fluorescence lifetime] imaging microscopy enables imaging of the intracellular temperature distribution at a high temperature resolution (0.18–0.58°C) and a high spatial resolution (~200 nm)."*

Finally, covisualization analysis of the intracellular temperature and mitochondria using the FPT and a fluorescent mitochondria indicator, respectively, revealed local thermogenesis around the mitochondria that released a surplus of energy in the form of heat via respiration. This local thermogenesis was accelerated when ATP synthesis was stalled by the uncoupling reagent 4-(trifluoromethoxy)phenylhydrazone [11]. The average temperature increase upon 4-(trifluoromethoxy)phenylhydrazone treatment was 1°C. This result clearly indicated that local thermogenesis around the mitochondria was caused by the respiratory function of this organelle. In live HeLa cells, we observed similar local heat production in the nucleus, around the centrosome and around the mitochondria [4]. Thus, this temperature distribution inside of the live cell was likely a general phenomenon, at least in mammalian cells.

Potential benefits of intracellular temperature imaging

What is the meaning of intracellular local heat production and what would be the benefits of monitoring this intracellular temperature distribution? As expected, there appears to be a good correlation between local thermogenesis and local metabolic activity. This correlation was clearly shown in the case of mitochondria, as discussed above. In addition, the observed cell cycle-dependent temperature changes in the cytoplasm and the local heat production around the centrosome could also be explained by the variation in the local metabolic activity. It has been shown that the activity of ribosomal synthesis varied depending on the cell cycle; the synthesis of ribosomal proteins starts at the end of mitosis, increases in the G1 phase and peaks in the G2 phase [12]. This increase in ribosomal protein production may result in active protein synthesis, which is correlated with an increase in the biochemical activity. It results in an increase in the cytoplasmic temperature in the G2 phase. Local thermogenesis around the centrosome area could be

caused by active ATP hydrolysis via molecular motor proteins [13]. The centrosome is a main site of microtubule nucleation [14] and thus, a high concentration of molecular motor proteins and highly activated ATP hydrolysis around the centrosome could result in localized heat production. This correlation supports the potential use of intracellular temperature imaging to monitor local cellular metabolism.

The monitoring of local cellular metabolism would be highly beneficial for the study of human diseases, as many diseases are characterized by their relationship with abnormal cellular metabolism. Most mitochondrial syndromes show an altered function of the respiratory chain [15] and thus may exhibit a different extent or an altered pattern of temperature increase around the mitochondria compared with healthy cells. In cancer cells, ATP is mainly produced via cytoplasmic glycolysis, whereas the energy production of healthy cells relies on mitochondrial oxidative phosphorylation [16]. This altered energy metabolism in cancer cells may potentially cause an altered temperature distribution. Using FPT-FLIM, malignancy progression may be quantitatively estimated. In addition, because many mitochondrial diseases, particularly milder ones, do not have established molecular diagnoses [15] and cancer cells are phenotypically and genetically very diverse, the imaging of altered metabolic activity using FPT-FLIM could serve as a rapid and efficient method of detecting malignant cells. Finally, intracellular temperature imaging using FPT-FLIM could also be beneficial for drug discovery. For example, the centrosome and mitochondria, which are organelles with local heat production, have been recognized as potential targets of cancer therapy [17,18]. Thus, monitoring heat production around these organelles using FPT-FLIM may serve as an efficient screening method and tool for the quantitative measurement of the effects of various drugs.

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