REVIEW

Fluorescence imaging for specific analysis of cancer cells

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Cancer is a serious disease that causes 25% of deaths in the developed countries. Significant impact on the cancer patients survival has early detection of this disease, therefore great attention is paid to its imaging. Fluorescence imaging represents powerful imaging method for the cell detection. For the successful detection of tumour cells, the specific targeting of fluorescence probes to the tumour tissue has a key role. Interesting materials enabling the imaging of tumour cells are fluorescence nanoparticles. For the accurate imaging, the NPs should be conjugated with targeting ligands and/or constructed as off-on probes.

Keywords: cancer cell; fluorescence; imaging; quantum dots

1. Introduction

Cancer is life-threatening disease, which causes nearly 7 million deaths every year worldwide and represents around 1 trillion dollars economic loss [1,2]. Cancer presents 25% of death caused in the developed countries [3]. But due to an early diagnosis and effective treatment, the mortality caused by this disease decreases and survival time increases [4]. The risk of dying from cancer decreased by 20% between 1991 and 2010 [3]. The cancer is diagnosed in the every third woman and every second man in the United States [5]. Cancer mortality in Czech Republic is about 20% [6,7]. Death rates continue to decline for all 4 major cancer sites (lung, colorectal, breast, and prostate), with lung cancer accounting for almost 40% of the total decline in men and breast cancer accounting for 34% of the total decline in women [4]. The most common causes of death are cancers of the lung, followed by colorectal, breast and stomach [7]. Early detection of cancer can significantly impact survival of cancer patients, so the regular screening is highly recommended [8-10].

2. Fluorescence imaging of cells

Optical methods are relatively cheap non-ionizing techniques based on the specific optical properties and are due to their variability, flexibility, specificity and sensitivity an important tool for non-invasive and objective diagnosis with still improving resolution [11-14]. Fluorescence imaging is the optical method based on the usage of fluorophores, the compounds, that can emit light after absorption of the appropriate wavelength light [15]. Fluorescence detection of cancer cells has the potential to be used in early cancer diagnosis [16]. The fluorescence of the dyes for imaging should be in the „tissue optical window" spectral ranges between 650 and 900 nm [17] or in the infrared spectrum [18]. The long wavelength (far-red to NIR) fluorescent probes are advantageous for *in vivo* bioimaging because of minimum photo-damage to biological samples, deep tissue penetration, and minimum interference from background autofluorescence by biomolecules in the living systems. Therefore great attention is paid in the development of new long wavelength fluorescent probes [19].

3. Fluorescent probes

As smart molecular probes, organic compounds such as fluorescein or rhodamin are widely studied [20,21] and as an universal, genetically encoded fluorescent label, green fluorescent protein, is applied [22]. Promising imaging tools represent inorganic compounds such as nanoparticles (NPs) [23,24]. The NPs accumulate in tumour cells by themselves, or they have to be targeted to the cancer cell surface molecules [25]. Interesting NPs with very good fluorescence properties are QDs [26-28]. They have broad absorption spectrum and a narrow emission spectrum and are photostable [29,30]. Fluorescence properties of QDs can be successfully exploit for imaging of tumour cells as well as in situ investigations of tumour tissue [31,32]. Because of the content of heavy metals, the toxicity of QDs is discussed [33]. CdTe QDs conjugated with antibodies were successfully used for the cancer cells detection [34,35].

Technical developments in fluorescence imaging have enabled recent translation into investigational human studies [36]. NIR fluorescence imaging is in some applications already used in the clinic. Indocyanine green (ICG) is NIR fluorophore used primarily in angiography [37]. ICG enable noninvasive imaging of the lymphatic vasculature and discrimination of malignant from benign breast lesions [36]. ICG is useful for the hepatocellular carcinoma detection [38]. ICG encapsulated within poly-(allylamine) hydrochloride chains cross-linked ionically with sodium phosphate (ICG-NCs) and functionalized with anti-HER2 can be used as theranostic agent for optical imaging and also for the photodestruction of ovarian cancers invitro [39].

Tryptophan is investigated as the key native marker in cells to determine the level of metastasis competence in breast cell lines. The ratio of fluorescence intensity is associated with aggressiveness of the cancer cells [40]. The higher content of tryptophan was detected in the advanced metastatic cancer cell lines against the moderate metastatic and non aggressive cell lines [41].

Cancer cells can reduce of innocuous silver salts and spontaneously generate silver nanoclusters (NCs), which has great fluorescence intensity. The formation of silver NCs also results in drastic reduction of tumour size and/or complete remission of the tumour [42]. Glutathione (GSH) can significantly and selectively enhance the fluorescence intensity of gold NCs. Gold NCs without GSH can selectively image the cancer cells. The liver cancer cells have much higher content of GSH, than other cell types. Therefore gold NCs enabled differentiation of cancer cells from normal ones [43].

For the targeting imaging, gold NCs conjugated with folic acid (FA) were utilized [44]. Folate receptor (FR) is overexpressed by a number of epithelial-derived tumors and minimally expressed in normal tissues. As FA is a high-affinity ligand to FR, and not produced endogenously, the FA-conjugated probes are specific for cancer cells imaging [45]. Folate-functionalized NPs are efficient imaging probes [46]. The overexpression of FR in 90–95% of epithelial ovarian cancers enabled the investigation of intraoperative tumor-specific fluorescence imaging in ovarian cancers surgery using an FR–targeted fluorescent probe [47]. Fluorescence imaging of cancer cells utilized in guidance surgery improves the tumour successful removal [48].

Low cytotoxic fluorescence probe comprises hydroxy-6-methyl-naphthalene-2-carbaldehyde was used for the detection of tyrosine kinase in cancer cells [49]. Lanthanum hexaboride LaB_6 NPs coated with a carbon-doped silica (C-SiO_2) shell to introduce a fluorescent property and improve stability and biocompatibility enabled fluorescent imaging and NIR-triggered photothermal therapy of cancer cells [50]. 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy--D-glucose (2-NBDG) was used as a tracer for detection of hypermetabolic circulating tumor cells (CTC) [51].

4. Turn off-on probes

For the sensitive and selective imaging without false positive results the „off-on“ fluorescence probes have been constructed. Polyethyleneimine-coated CdS/ZnS QDs (PEI-CdS/ZnS QDs) with the electrostaticly absorbed FA were turned off. In the presence of FR, the FA was desorbed and the fluorescence signal of QDs

was detected [45]. The fluorescence-quenching platform based on the biomineralized HAp (hydroxyapatite) has been also internalized for cancer cell detection [52]. Protein LAPTM4B is characteristic for a large number of cancer cells. Small peptide IHGHHIISVG (AP2H) is a targeting ligand for the LAPTM4B and therefore could be used for targeting the fluorescence probe to the cancer cells. Huang et al. constructed the turn-on probe consisted of the peptide and tetraphenylethylene (TPE), an aggregation-induced emission (AIE) fluorophore [21]. Another turn-on probe was designed via hydrogen-bond interaction between FA and carbon dots (CDs) with the passivating agent - poly(acrylate sodium) (PAAS). This probe could be used in the fluorescence-assisted surgical resection and real-time monitoring of the cells [16]. Acid phosphatase (ACP) is an important biomarker and indicator of prostate cancer. An „off-on" probe for ACP detection consisted of a near-infrared mercaptopropionic acid (MPA)--capped CuInS2 QDs [53].

5.Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) with centromeric probes is used to detect chromosomal instability (CIN), which is observed in many cancers. The chromosome doubling could influence the tumours heterogeneity [54]. A multi-gene fluorescence *in situ* hybridization (M-FISH) was used to investigate gene copy number aberrations (CNAs) and it was found, that the gene copy number aberrations (CNAs) of cell cycle-regulated genes can be significant for prognosis in young breast cancer patients [55]. FISH is a useful technique for ALK gene rearrangement analysis and specification of the type of gene irregularities. ALK gene examination could be applied in histological and also in cytological samples. FISH was utilized in the FISH of samples from NSCLC patients [56].

6. Conclusions

Cancer is one of the most serious diseases worldwide. The most important for its succesfull treatment is early detection of tumour in the body. Methods for the fast and easy tumour detection are widely studied. Interesting application represents fluorescence imaging. There are many possibilities of the fluorescence imaging of tumour cells. The most important for the use of fluorescence imaging in human medicine will be the necessity of the construction of the highly sensitive fluorescence detectors to detect the fluorophores deeply in the body.

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Conflicts of Interest

The authors declare no conflict of interest.

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