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Quantitative determination of VEGF165 in cell culture medium by aptamer sandwich based chemiluminescence assay

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Abstract

In this work, we have developed a sensitive and selective chemiluminescence (CL) assay for vascular endothelial growth factor (VEGF165) quantitative detection based on two specific VEGF165 binding aptamers (Apt). VEGF is a predominant biomarker in cancer angiogenesis, and sensitive detection method of VEGF are highly demanded for both academic study and clinical diagnosis of multiple cancers. In our experiment, VEGF165 was captured in a sandwich structure assembled by two binding aptamers, one capture aptamer was immobilized on streptavidin-coated magnetic beads (MBs) and another VEGF-binding aptamer was labeled by biotin for further phosphatase conjunction. After Apt-VEGF-Apt sandwich was formed on MBs surface, alkaline phosphatase (ALP) was modified to the second aptamer to catalyze CL reaction. By applying 4-methoxy-4-(3-phosphatephenyl)-spiro-(1,2-dioxetane-3,2-adamantane) (AMPPD) as CL substrate, strong signal intensity was achieved. VEGF165 content as low as 1ng/mL was detected in standard spiked samples by our assay, and linear range of working curve was confirmed from 1 to 20 ng/mL. Then our method was successfully applied for cell culture medium analysis and on-chip hypoxic HepG2-HUVEC co-culture model study with excellent accuracy equal to ELISA Kit. Our developed assay demonstrated an outstanding performance in