

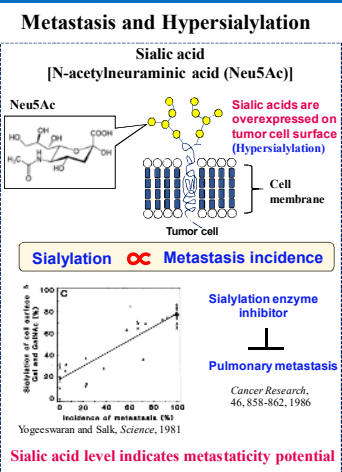
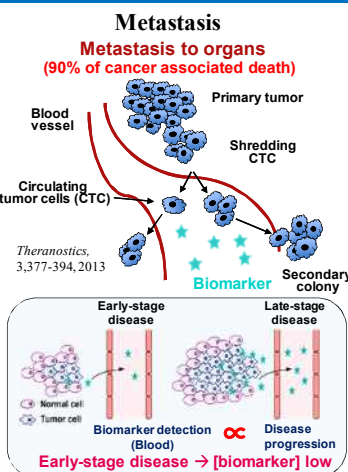
がん細胞およびがん細胞特異的粒子分泌物のSERSを用いる選択的高感度検出に関する調査研究

Detection of hypersialyated metastatic cancers by surface enhanced Raman scattering

概要

- [1] Application of phenyl-boronic acid-installed PEGylated (APBA-PEG-*b*-PAMA) gold nanoparticles (GNP) coupled with Toluidine blue O (T/BA-GNPs) as surface enhanced Raman scattering (SERS) probes to target surface hypersialyated (N-acetylneuraminic acid, Neu5Ac) metastatic cancer cells and tumors tissue explants.
- [2] Reactive oxygen species (ROS)-mediated abrogation of sialylation pathway in cancer cell lines by nitroxide-radical containing nanoparticle (RNP)

INTRODUCTION



This study

Synthesis of SERS probe APBA-PEG-*b*-PAMA targeting sialic acid

[A] APBA-PEG-*b*-PAMA synthesis scheme

Acetal-PEG-*b*-PAMA → Deprotection of acetal base in acidic condition → APBA-PEG-PAMA

APBA-PEG-PAMA + T/BA-GNPs → Specific SERS signal of T/BA-GNPs aggregates on cell surfaces

Colon-26 cell surface

SERS measurement

Count (x 10⁴) vs Raman Shift (cm⁻¹)

890 cm⁻¹, 1432 cm⁻¹, 1620 cm⁻¹

ring C-C stretching vibration or C-N stretches
C-N-C asymmetric stretching motion, C-S-C asymmetric stretching motion or ring C-C stretching vibration
C-C stretching vibration, bending deformation vibration of NH₂

Z-Average: 53.34 nm, PDI: 0.162

RESULTS

[1] SERS intensity correlates with metastatic potential in breast cancer cell lines

SERS measurement protocol

Colon-26 (5X10⁴ cells/dish) → Incubation (24 h) → Fixation → Incubation (30 min) → Wash → T/BA-GNP addition → Incubation (30 min) → Wash → Dry completely → Raman spectrum measurement

[A] Absorption spectra

[B] SERS spectra

[C] Quantification of SERS spectra

[D] Sialic acid quantification (commercially available kit)

Substantial count (x 10⁴)

Total sialic acid level (% to MDA-MB231)

MDA-MB231, MCF-7, Colon-26

No visible change detected by UV-vis spectroscopy

[2] ROS scavenging nitroxide-radical containing nanoparticles (RNPs) as anti-metastatic candidate

Hydrophilic (PEG) / Hydrophobic (PMNT, PMOT)

Self assembly → RNP^o (40 nm)

ROS scavenger: TEMPO

ROS → NF-κB → α2,6-sialyltransferase → ST6GAL1 → Transfer of Neu5Ac onto the substrate galactosamine (GAL)

RNP^o → ROS → Cancer cell Migration + Metastasis

RNP^o → ROS → Acute kidney injury, Cerebral ischemia-reperfusion injury, Alzheimer's disease, Ulcerative Colitis, Colon Cancer

[2.1] RNPs inhibit sialylation via ROS-mediated suppression of sialyltransferase

In vitro

MDA-MB-231 (5X10⁴ cells/dish) → Incubation (24 h) → Incubation (48 h) → Fixation → T/BA-GNP addition → Raman spectrum measurement

[A] RNP → Sialylation-SERS intensity

[B] RNP → ROS

[C] RNP → NF-κB → α2,6-sialyltransferase

Substantial count (x 10⁴)

ROS level

TEMPO (mM)

α2,6-sialyltransferase

β-actin

NF-κB

RNP^o, RNPⁿ

In vivo

MDA-MB231 cells (4-6 weeks C57 female mice) → Day 0 → Day 36 → Tissue extraction → Slicing → Fixation → T/BA-GNP addition → Raman spectra

Healthy, Tumor, RNPⁿ-treated

Count (x 10⁴)

Substantial count (x 10⁴)

Healthy, Tumor, RNPⁿ

CONCLUSION

T/BA-GNP-SERS: Potential cytodiagnostic system

ROS-scavenging RNPs: Potential anti-metastatic agent

T/BA-GNP based SERS system

RNPs inhibits sialylation

SERS probe (GNP) + Toluidine Blue O

Normal cell vs Hypersialyated tumor cell

RNP^o → ROS → NF-κB → α2,6-sialyltransferase → Hypersialylation → Metastasis

SERS intensity in tissue explants