MRI visualization of hyaluronidase in ovarian cancer cells

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Introduction:

Hyaluronan (HA) is a major constituent of the extracellular matrix. It plays a role in maintenance of the architecture of normal tissues, cell adhesion and migration, and angiogenesis. HA also mediate adhesion of cancer cells, including metastatic implantation of ovarian carcinoma in the peritoneum, through interaction with the CD44 receptor, expressed on the surface of the tumor cells. High molecular weight HA is antiangiogenic, but its degradation by hyaluronidase (Hyal) generates pro-angiogenic breakdown products. The activity of Hyal is elevated in tumors (*1, 2*), and correlated with aggressiveness and invasiveness of ovarian cancer. Thus, ovarian carcinoma cells adhere to HA and degrade it by secretion of Hyal, thereby converting anti-angiogenic high MW HA into the low MW pro-angiogenic breakdown products, promoting tumor growth and metastasis. Several methods are being used for the detection of hyaluronidase (*3-6*), but none of these methods can be applied in-situ for non-invasive imaging. The goal of this work was to develop a novel method for detection of hyaluronidase by MRI.

Materials and methods:

Contrast materials: HA-GdDTPA was synthesized as described (*7*) and was covalently bound to agarose beads.
Hyaluronidase: Hyaluronidase expression, activity and secretion were studied in OVCAR-3 and ES-2 human epithelial ovarian carcinoma cell lines using RT-PCR, western blot and particle exclusion assay (*8*).
MRI studies: horizontal 4.7T Biospec Bruker spectrometer was used. Determination of R_2: multi echo spin echo sequence with 8 echo times (TR= 2000 ms TE 10-80 ms, 2 averages, FOV 1X1 cm, slice thickness 1 mm, matrix 128 X 128, SW=50,000Hz).
Analysis of the MR data: R_2 maps were generated by pixel-by-pixel non-linear single exponential fit using Matlab.

Results:

HA-GdDTPA-beads

The R_2 and R_1 relaxivities of the HA-GdDTPA-beads (fig 1) was measured to be 25 and 11 $mM^{-1}S^{-1}$ per Gd respectively.

The beads reaction with Hyaluronidase caused significant changes in the relaxivity of the surrounding media (n=6; 2 tailed unpaired t-test p=0.009; fig 2).

Hyaluronidase activity in two ovarian carcinoma cell lines

High expression and activity of hyaluronidase (Hyal-2) in the culture media was detected by western blot and particle exclusion assay for ES-2 cells, while expression and activity were low for OVCAR-3 cells.

Contrast enhancement by hyaluronidase secreted by ovarian carcinoma

Addition of conditioned media from ES-2 cells to HA-GdDTPA-beads significantly increased R_2, while conditioned medium from OVCAR-3 cells had only small effect (n=3; 2 tailed unpaired t-test p=0.005; fig 3). Thus the MRI detected changes in water relaxation, correlated with the expression and biological activity measurements of hyaluronidase.

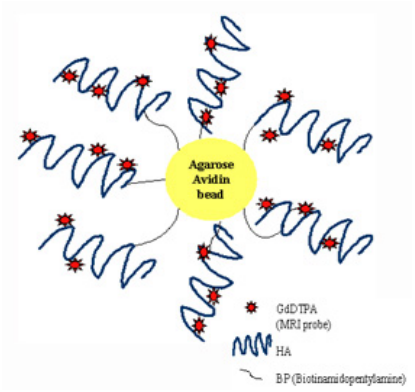

Figure 1: Scheme of the contrast material HA-GdDTPA-beads for MRI detection of hyaluronidase. Hyaluronan (HA) was conjugated with GdDTPA, and the complex was attached to agarose-avidin beads via biotinamidopentylamine (BP) as a linker. Degradation of the hyaluronan contrast material by hyaluronidase to low molecular weight fragments was expected to alter R_2 relaxivity and thus change the contrast in MR images.

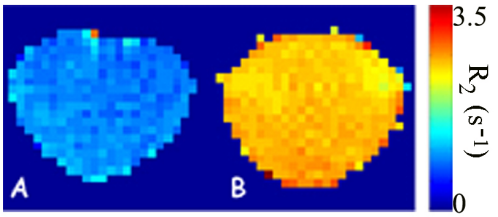

Fig 2: Color-coded map of R_2 relaxation rate of water for a suspension of 1mg/ml HA-GdDTPA-beads. A) Control suspension in water. B) Water suspension in the presence of hyaluronidase (1mg/ml). R_2 was measured after incubation with the contrast material for 15-30 min at 37ºC

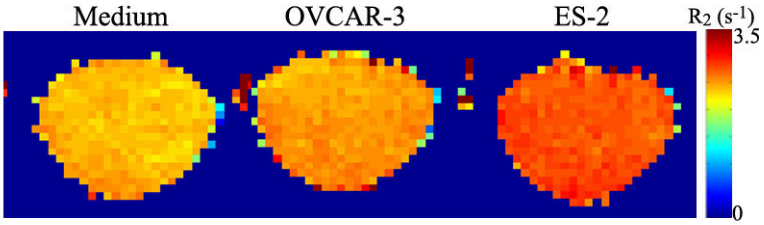

Fig 3: MRI detection of Hyaluronidase secreted by ovarian carcinoma cells. A representative color-coded map of R_2 relaxation was derived for HA-GdDTPA-beads suspended in fresh medium (left), or in conditioned medium collected from OVCAR-3 (center) or ES2-2 (right) cells.

Conclusions:

- Hyaluronidase increased R_2 relaxation rates of the HA-GdDTPA-beads; hence HA-GdDTPA enveloped beads enabled detection of hyaluronidase in-situ by MRI. The beads were sensitive to various physiological concentrations of hyaluronidase.
- The novel approach of delivering the beads to the investigated cells, allows the benefit of rapid and spatial detection for future non invasive in vivo use.

References:

1. E. A. Balazs, J. Von Euler, Cancer Res 12, 326-9 (1952). **2** L. B. Cobbin, S. E. Dicker, J Physiol 163, 168-74 (1962). **3**. A. Dorfman, J. Biol. Chem 172, 377-87 (1948). **4**. G. I. Frost, R. Stern, Anal Biochem 251, 263-9 (1997). **5**. K. P. Vercruysse, A. R. Lauwers, J. M. Demeester, J Chromatogr B Biomed Appl 656, 179-90 (1994). **6**. J. A. Cramer, L. C. Bailey, Anal Biochem 196, 183-91 (1991). **7**. S. Gouin, M. V. V. Grayeb, F. M. Winnik, Macromolecular Symposia 186, 105-10 (2002). **8**. W. Knudson, E. Bartnik, C. B. Knudson, Proc Natl Acad Sci U S A 90, 4003-7 (1993).

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