

## MRI Detection of Hyaluronidase Secreted by Ovarian Carcinoma Cells

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### Abstract

Hyaluronan (HA) and hyaluronidase (HYAL), play an important role in many pathological and biological processes. Ovarian cancer cells secrete HYAL which could enable digestion of the HA barrier allowing invasion to neighboring tissues. HYAL also converts anti-angiogenic high molecular weight (MW) HA into low MW fragments, which can also promote angiogenesis, thereby facilitating dissemination of metastases. The goal of this work was to develop a novel method for detection of HYAL activity by MRI. For that purpose we labeled HA with Gd-DTPA (Gd-Diethylene triamine pentaacetic acid) and bound it to non-toxic beads. By addition of this contrast agent to media of various ovarian carcinoma cell lines that secrete HYAL, we were able to detect pronounced alternations in  $R_1$  and  $R_2$  relaxation rates. We propose that this probe can be developed for non-invasive *in vivo* MRI mapping of HYAL activity.

### Keywords

MRI, hyaluronidase, contrast enhancement, ovarian cancer

### Introduction

Hyaluronan (HA) is involved in processes that require cell motility including wound healing, inflammation and angiogenesis, as well as cancer invasion and metastasis. HYAL degrades antiangiogenic high MW HA to low MW products, which stimulate endothelial-cell proliferation and neovascularization. Secretion of HYAL contributes to cancer invasiveness, and was correlated with metastasis in ovarian carcinoma (1). Moreover, in a model of peritoneal cavity, treatment with HYAL containing conditioned media increased the adhesion of ovarian cancer cells to a mesothelial monolayer (2).

GdDTPA is a non-toxic chelate of gadolinium, which is widely applied as an MRI contrast material. GdDTPA increases the magnetic relaxation rate of water ( $R_1=1/T_1$  or  $R_2=1/T_2$ ) and by that increases the brightness of  $T_1$  weighted MRI by increasing signal intensity or decreases signal intensity in  $T_2$  weighted images. We bound GdDTPA to high MW HA to generate a new biologically active MRI contrast material that would induce alterations in  $R_1$  and  $R_2$  as a result of HYAL activity.

The methods available today for detection of HYAL activity require extraction of HYAL from the tissue in order to be subjected to the assay, and none of these methods can be applied *in-situ*. Detection of HYAL activity *in-vivo* requires a sensitive non-invasive technique. Therefore, we attempted to develop a novel method for detection of HYAL activity by MRI.

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### Materials and Methods

**Contrast material:** HA-GdDTPA was synthesized as described (3). The relaxivity was measured on a 4.7 T Bruker spectrometer to be  $5.3 \text{ mM}^{-1}\text{s}^{-1}$  per Gd. Avidin linked agarose beads (Sigma, St. Louis, Missouri), were saturated with 5-(biotinamido)pentylamine (BP, Pierce, Illinois). Carboxyl groups of HA were activated by EDC (N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride) and reacted with BP.

**Cell culture:** Human ovarian carcinoma cell lines ES2 and OVCAR and rat chondrocytes (Prochon Biotec Ltd.) were received from Prof. Benny Geiger, Weizmann institute.

**MRI studies:**  $R_1$  and  $R_2$  values of HA-GdDTPA-beads were measured before and after addition of HYAL or conditioned medium taken from ES2 or OVCAR cells. MRI measurements were performed on a 400 MHz wide-bore DMX Bruker spectrometer.

**Analysis of the MR data:**  $R_1$  and  $R_2$  maps were generated from the obtained MRI data by monoexponential fitting using Matlab.

**Particle exclusion assay for HYAL activity:** Rat chondrocytes that secrete HA were seeded (in low concentration) and incubated either in fresh medium (negative control), medium with HYAL (positive control) or conditioned medium from ovarian carcinomas ES2 or OVCAR. The medium was then replaced by human erythrocytes (Sigma) in DMEM. Samples were viewed by microscope, and the area of the remaining HA was measured.

### Results

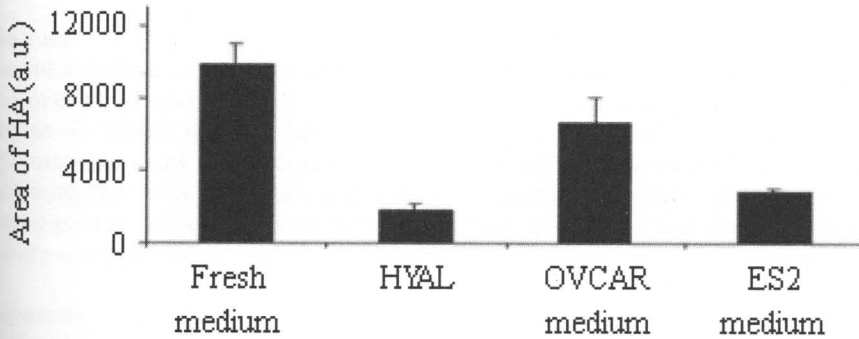
**Detection of HYAL activity using particle exclusion assay:** Particle exclusion assay was used to measure the secretion of HYAL by OVCAR and ES2 ovarian carcinoma cell lines. The degree of secretion and activation of HYAL was estimated from changes in thickness of the chondrocyte HA layer (Figure 1). The area of HA decreased by 4.5 fold after treatment with HYAL and by 3 fold after treatment with ES2 conditioned medium, while treatment with OVCAR medium decreased the HA layer only by 0.8 fold. Thus conditioned medium derived from ES2 cells showed high levels of HYAL activity, while OVCAR medium showed moderate activity. The areas of HA layer were measured after treatment with fresh medium, HYAL containing medium or conditioned medium from human ovarian carcinoma cells, OVCAR and ES2.

**MRI detection of HYAL activity using HA-GdDTPA beads:** HA-GdDTPA beads were inserted into the NMR tubes with various media conditioned by ovarian carcinoma cell lines, OVCAR and ES2 (or by fresh medium).  $R_2$  relaxation rates were measured (Figure 2). Significant differences in  $R_2$  between fresh medium OVCAR and ES2 medium were found. This suggests the capability of the beads to provide a molecular imaging probe for detection of HYAL activity by MRI.

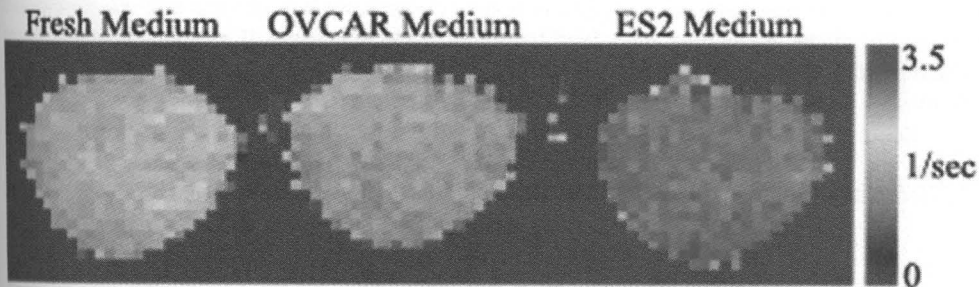
### Discussion

HA synthesis, binding and degradation have important roles in ovarian carcinoma adhesion, invasion and in regulation of angiogenesis. We show here a single step, *in-situ* assay for detection of HYAL activity, which potentially could be adapted for non-invasive *in-vivo* imaging by MRI. Using beads enveloped with HA-GdDTPA, we were able to detect significant changes in  $R_1$  and  $R_2$  relaxation rates after addition of conditioned medium taken from ovarian carcinoma cell lines. Secretion of HYAL by these cell lines was confirmed by exclusion of erythrocytes. The high level of HYAL activity detected in ES2 medium by

exclusion assay was consistent with the marked change in  $R_2$  relaxation rate as detected by MRI. OVCAR medium that was found by the exclusion assay to have less HYAL activity also showed a reduced  $R_2$  effect. This novel approach could allow detection of HYAL by delivery of the beads to the investigated tissue for future *in-vivo* use.



**Figure 1.** HYAL activity secreted by human ovarian carcinoma cells measured by particle exclusion assay. Erythrocytes were added to culture plates to allow determination of the area of HA layer around chondrocytes. The areas of HA layer were measured after treatment with fresh medium, HYAL containing medium or conditioned medium from human ovarian carcinoma cells, OVCAR and ES2.



**Figure 2.** MRI analysis of hyaluronidase activity.  $R_2$  maps were determined from MR images after addition of conditioned medium from human ovarian carcinomas, OVCAR and ES2, or fresh medium to HA-GdDTPA beads.

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