Identification of Gelatinases involved in the Rous sarcoma Virus-induced Tumors in Chicks as Prognostic Markers

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Abstract

The present work is undertaken to study the expression of levels of gelatinases in tumorogenesis by Rous sarcoma virus(RSV) in layer chicks and explored the possibility of using gelatinases as potential biological markers in metastatic neoplasms. Two days old chicks (40) were divided into two groups (Gp I and Gp II). Gp-I (20) treated with Rous sarcoma virus for tumor induction. The Gp II (control) was inoculated with RPMI-1640. Tumors appeared earliest by three days post infection with RSV and were progressive leading to mortality of birds by twenty eight days. Distant tumors were observed in liver, heart, lung, and kidney on post mortem. A prominent band of gelatinase of around 75 kDa was detected in plasma of infected chicks by gelatin zymography. Results indicate over expression of gelatinases and are leaked into plasma on Rous sarcoma virus infection. Expression of gelatinases in primary tumors, metastasized liver, heart, lung and kidney and corresponding tissues in healthy control chicks was determined by RT-PCR analysis. Over expression of gelatinase gene was observed in metastaic tissues and primary tumors than control. The described assays could be used as a prognostic assay method for detection of proteases in metastatic neoplasms of animals.
Keywords: Tumor, Chick, Prognostic Marker, Gelatinases, Virus,

Introduction

Gelatinases or matrix metalloproteinases are zinc dependent endopeptidases involved in the degradation of the extracellular matrix (Woessner, 1991). The degradation of the extracellular matrix by malignant cells is intrinsic to the invasion and progression of cancer to remote organs. Proteases are directly involved in cancer spread; therefore have the potential to be new prognostic markers in cancer (Duffy, 1992). Consistent with their role in cancer progression, high levels of proteases have been found to correlate with poor prognosis in cats with malignancies (Jankawaski et al., 2002).

At present, there are no reports of use of proteases as prognostic markers in animal neaoplastic diseases. A novel 75-kDa avian counterpart of gelatinase B from a chicken macrophage cell line, HD11 was characterized and purified (Hahn-Dantona, 2000). The study indicates the involvement of gelatinase of 75 kDa in degradation of extracellular matrix in physiological and pathological processes. RSV transformed chicken emryofiroblasts constitute best model for studying oncogenic transformation and matrix degradation and cancer invasion. (Alexander et al., 1996). This study presents the methodology to analyze the circulating plasma gelatinase levels and tissue gelatinase expression using mRNA and explored the possibility of using gelatinases as potential biological markers in various metastatic neoplasms of animals.

Materials and Methods

Apparently healthy white leg horn day old layer chicks (n=40) were obtained from the experimental hatchery of Central Avian Research Institute, Izatnagar and kept in a disinfected room under standard managemental and hygienic conditions. Chicks were divided into two groups (Gp I and Gp II) of 20 each. Gp I (Two days old) chicks were inoculated with 0.2 ml of Rous sarcoma virus filtrate obtained from Avian Diseases Division, Indian Veterinary Research Institute Izatnagar, intradermally at the wing web. The Gp II (control) was inoculated with RPMI-1640 similarly.

Tumor growth was monitored everyday. Blood was collected aseptically by jugular venipuncture in test tubes containing heparin @10-20 U/ml of blood from three chicks of Gp I and Gp II randomly on 20th day of post inoculation. Plasma was prepared and kept in -20°C until use. Protein concentrations of plasma samples were determined by the Folin-Ciocalteau method (Lowry et al., 1951). During the course of the experiment, pieces of primary tumor, and visceral organs like liver, kidney, heart, and lungs were collected directly in liquid nitrogen from the dead chicks.

Plasma proteolytic (Gelatinase) activity was determined by modified multigel SDS-PAGE Gelatin Zymography. In this study we designed a method to detect proteases and their putative molecular weight based on SDS-PAGE technique(Sushanta Kumar Saha et.al., 2003) where in two gels, one polyacrylamide gel impregnated with a protein substrate, gelatin which is degraded by the proteases(Hussein and Dowdle,1980) and other normal SDS-polyacrylamide gel on which same quantity of sample and molecular markers were subjected. Briefly, samples were diluted in SDS sample buffer in the absence of reducing agents and electrophoresed on 10% SDS-polyacrylamide gels co-polymerized with 0.1% gelatin in one gel and only 10% SDS-polyacrylamide gel in another. Gelatinases present in plasma degrade the gelatin matrix after incubation in suitable buffer, leaving a clear band after staining the gel for protein.

RT-PCR was used to characterize the expression a level of gelatinases in various tissues in which metastasis has taken place. Briefly, total cell RNA extraction was done using the acid guanidinium isothiocyanate method. One microgram of total RNA, determined by a UV spectrophotometer was reverse transcribed to cDNA in a reaction mixture containing all appropriate deoxynucleoside triphosphates (dNTPs) and MuLV reverse transcriptase. PCR reactions were performed by standard protocol with 2 µl of reverse transcriptase product serving as template DNA (synthesized cDNA), appropriate dNTPs, and 20 pmol each of forward primer (5?CGGCGGCATTCAAGGT GTGGAGT3?) and reverse primer (5? TGAAGGGGA AGACGCAGGGAGACC 3?).

Results and Discussion

In this study we evaluated the clinical usefulness of plasma gelatinases as a diagnostic and prognostic biomarker in chicks undergoing progressive RVS infection which has much significant economic importance. Primary tumor formations at the site of virus inoculation was observed in chicks earliest by three days and latest by seven days post infection and were progressive leading to mortality of birds by twenty eight days. Progressive sarcomas lead to the death of the infected chicks by twenty nine to thirty days. Visceral tumors in various organs like heart, lung liver and kidney were observed on twenty-eight days post infection. Grossly visible progressively growing metastatic foci varying in size were mostly the cause of fatality. It was found that sarcomas localized to visceral organs frequently arise following wing web inoculation of RSV. (Halpern, et al., 1996). Plasma gelatinase activity of infected chicks showed prominent band indicating more concentration of gelatinases. The putative molecular weight of circulating gelatinase was shown to be around 75 kDa as determined by gel documentation system.

Gelatin zymography is a very sensitive and standard electrophoretic method for detection of gelatinases and provide information about active gelatinases. There are many reports of potential usefulness of gelatin zymography in the follow-up and in the prognosis of human neoplastic cases. Two gelatinases, gelatinase A (72kDa) and gelatinase B (92kDa) have been reported to be associated with malignant tumor progression and metastasis in human ovarian cancer (Barbara et al., 2001). RT-PCR analysis showed consistent differences in mRNA expression levels in treated versus control groups (Fig.1). RT-PCR can sensitively detect individual mRNA species. Consistent differences in mRNA expression levels found in treated versus control groups showing positive correlation with high metastatic activity in RSV infection. It is clear that the gelatinases have important roles in spread of distant tumors leading to metastasis and death of birds in RSV infection In conclusion; increased plasma gelatinase could be related to fatal metastatic process in birds with non regressing RSV infection.

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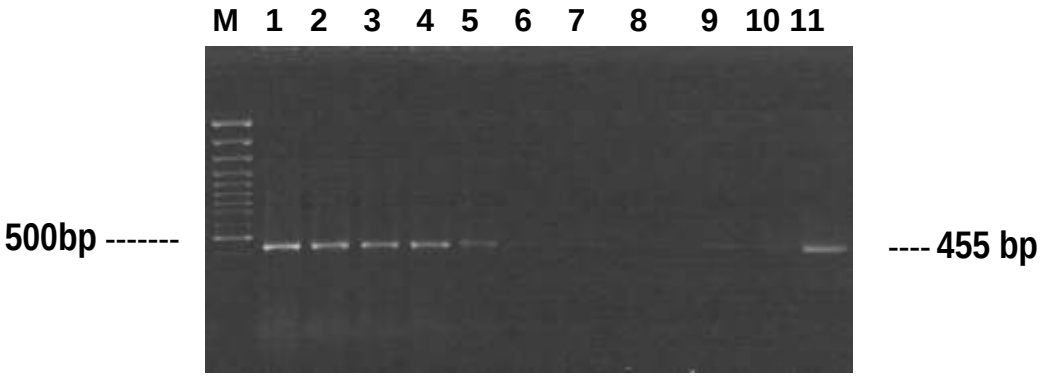

Figure-1. RT-PCR Analysis of galatinase mRNA
Lane: 1, 2, 3, 4, 5 - Liver, Kidney, Lung, Heart & Muscle from treated chicks.
Lane: 6, 7, 8, 9, 10 – Liver, Kidney, Lung, Heart & Muscle from control chicks.
Lane: 11. Primary Tumor (wing web).

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