Identification of Secreted Proteins from Ionizing Radiation-Induced Senescent MCF7 Cells Using Comparative Proteomics

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1. Introduction

Cellular senescence was first described by Hayflick and Moorhead in 1961 who observed that cultures of normal human fibroblasts had a limited replicative potential and eventually became irreversibly arrest [1]. The majority of senescent cells assume a characteristic flattened and enlarged morphological change, senescence associated β–galactosidase positivity and over the years a large number of molecular phenotypes have been described, such as changes in gene expression, protein processing and chromatin organization [2-4]. In contrast to apoptosis, senescence does not destroy the cells but leaves them metabolically and synthetically active and therefore able to affect their microenvironment. In particular, senescent fibroblasts and some cancer cells were found to secrete proteins with known or putative tumor-promoting functions such as growth factors or proteolytic enzymes [5]. However, the knowledge about secreted proteins from senescent tumor cells and their functions to surrounding cells is still lacking. In this study, changes of senescence-associated secretory protein expression profile were observed in MCF7 human breast cancer cells exposed to gamma-ray radiation using two dimensional electrophoresis. Also, we identified up-regulated secretory proteins during ionizing radiation-induced cellular senescence.

2. Methods and Results

2.1 The Effects of Senescence-Associated Secretory Proteins

Human breast carcinoma MCF 7 cells were grown in conditioned media from senescent or presenescent MCF7 cells for 24 h. Cell proliferation was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetraz-lium bromide (MTT) colorimetric growth assay. The test is based on the ability of mitochondrial dehydrogenase in viable cells to convert MTT reagent (Sigma) into a soluble blue formazan dye. Cells were plated in 35 ㎜ plates at the density of 1×10^4 cells/ well and cultured for the indicated time intervals. At the end of each incubation, the medium in each well was replaced with fresh medium containing 100 ㎕ of MTT, and the plate was incubated for 2 h. After incubation, cells were lysed with solubilization solution and the relative amount of formazan were determined by measuring the absorbance at 595 ㎚ using ELISA reader (Molecular Devices, spectra max M2).

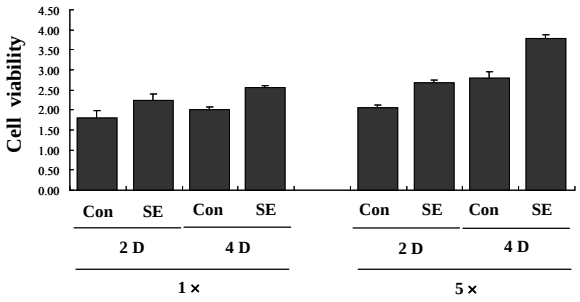

Fig. 1. Senescence-associated secretomes increase cell growth rates of neighboring tumor cells.

Invasion assays were performed using modified Boyden Chambers with 8-microcon pore filter inserts for 12-well plates (BD Bioscience). Filters were coated with basement membrane extract (Matrigel, BD Bioscience). Cells were added to the upper chamber in serum-free medium, while the lower chamber was filled with senescent or presenescent conditioned medium. After 24 h, cells on the underside of the filter were fixed with Diff Quick. Cells on the underside of the filter were counted using light microscopy. For wound healing assay, at 24 h after seeding the cells, a linear wound was generated on the confluent monolayers by scraping with a pipette chip. Unattached cells were washed off with agitation. Cells were photographed at the same point on a grid 24 h later. Each line was plated and wounded in triplicate.

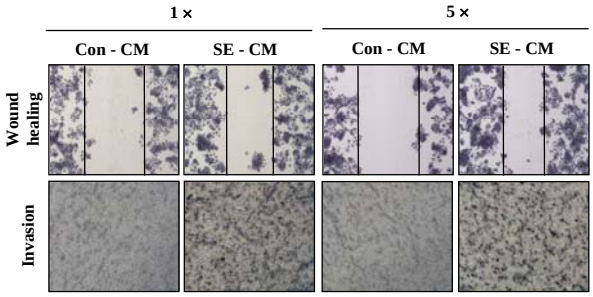

Fig. 2. Senescence-associated secretomes stimulate invasion and migration of neighboring tumor cells.

2.2 Identification of Senescence-Associated Secretory Proteins Using by Two Dimensional Electrophoresis

To compare secretory proteins from senescent and presenescent MCF7 cells, proteins in conditioned media were concentrated to 250 ㎍ in a buffer containing 8 M Urea, 2% CHAPS, 2% IPG buffer, and 0.002% of bromophenol blue. Samples were loaded using the passive rehydration method for a minimum of 16 h. Rehydrated strips were focused at a temperature of 20 °C using the IPGphor IEF apparatus. The strips were brought to room temperature and equilibrated for 15 min each to reduce and alkylate the proteins. The equilibration buffer contained 6 M Urea, 2 M Thiourea, 50 mM Tris-Cl pH 8.8, 30% glycerol, 2% SDS, and 0.002% bromophenol blue with 2% DTT for the first step, and 2.5% iodoacetamide replaced DTT for the second step. Strips were then placed on top of a 12.5% polyacrylamide gel and sealed with 0.5% agarose dissolved in electrophoresis buffer. The DALT six apparatus was used for 2DE a constant temperature of 17 °C. For coomasie blue staining, place the gel in stain solution (0.2% Brilliant Blue R250 in 20% methanol, 0.5% acetic acid) for 60 minutes. And then, pour off the stain solution into a hazardous waste container and rinse the gel briefly with distilled water. For distaining, place the gel in 100 ml 30% methanol. Replace with fresh destain solution every half hour. After staining we were used the top of tips for spotting, and the spot were identified from MALDI-TOF MS.

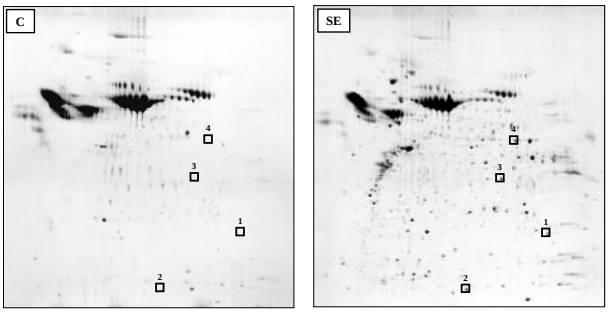

Fig. 3. Senescence -associated secretomes compared with presenescent secretomes by two dimensional electrophoresis.

Table. 1. List of identified senescence-associated secreted proteins from MCF 7 cells by MALDI-TOF MS

Spot no.	Acc. number	MW (KDa)	Matched Peptides	IR-induced Expression
1	CH471126.1	61929	11	Up
2	NM002506.2	21044	10	Up
3	AB019691.1	451560	25	Up
4	AK222517.1	47111	19	Up

3. Conclusions

In this study, secreted proteins from IR-induced senescent MCF7 cells were analyzed by two dimensional electrophoresis and MALDI-TOF MS. Our data evidenced that senescent cancer cells secreted a lot of secretomes which affect to tumor microenvironments and they stimulated not only neighboring cell growth but also invasion and migration rates. Our results propose that senescence-associated secretome could be the principal targets that have potentially the effects on neighboring cell growth-stimulation.

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