EVALUATION OF GLYCOSAMINOGLYCAN EFFECTS TOWARDS CISPLATIN CHEMOSENSITIVITY ON BREAST CANCER CELLS

Sen Y.P.¹ and Yip G.W.²

Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore
MD10, 4 Medical Drive, Singapore 117597

ABSTRACT

Breast cancer is a major cancer mortality cause especially in women worldwide. New approaches like glycosaminoglycans’ effects towards chemotherapy drug chemosensitivity are researched as an improved therapeutic way to combat this disease. Chondroitin sulfate (CS)/Dermatan sulfate (DS) are glycosaminoglycans (GAGs) found abundantly and naturally in our body system and at times used as treatment for diseases like arthritis. However, these GAGs have been reported to promote metastatic growth of breast cancer cells. The studies carried out in this project have shown that the endogenous and exogenous CS/DS influence breast cancer cell proliferation level in cisplatin (CDDP) presence in different ways; most interestingly DS was found to make cells more susceptible to CDDP effect. In addition, the experiments have also confirmed the importance of endogenous CS/DS and their sulfation sites in enhancing cell growth.

INTRODUCTION

Breast cancer is the most common cancer affecting women. According to the most recent report, approximately 1.2 million females are newly diagnosed of breast cancer annually and more than ½ million deaths per year are caused by this disease globally. (WHO Cancer, 2005) With breast cancer diagnosis on the rise, more effective treatments with minimal side effects are being researched to counter the disease. For metastatic breast cancer, one of the more common treatments is chemotherapy. However, breast cancer therapies should not only be limited to physical (surgery) or chemical (chemotherapy) methods alone. Biological factors (naturally found in our body or administered orally or intravenously) can provide new clues in cancer progression and suggest new therapeutic ways against cancer diseases. Several findings have reported that CS/DS, a major class of GAGs present in various tissues, is associated in promoting progression of malignant tumors. Interference with this GAG expression/function can result in the inhibition of malignant process of several cancers. (Yip, 2006)

CS/DS are sulfated GAGs composed of linear repeating units (N-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA)). CSs are classified into 5 types: CSA, CSC, CSD, CSE and CSO. DS is a modified version of the CS chain which undergoes epimerization at C5 carbon of GlcA forming iduronic acid (IdoA). (Silbert, 2002) With the idea that CS/DS enhances the cells’ metastatic potential, one should also focus on studying CS/DS effects with a chemotherapeutic drug which is usually administered to a patient diagnosed with metastatic

¹ Student
² Doctor
breast cancer. Hence, we seek to investigate endogenous and exogenous CS/DS effects towards a drug chemosensitivity (in particular CDDP) on MDA-MB-231 cell proliferation.

MATERIALS AND METHODS

MDA-MB-231 metastatic breast cancer cell line, CDDP (at LD\textsubscript{50} towards the cells) and various CS/DS were used in this study. MTS assay was used to obtain the cell proliferation level. The principle behind MTS assay is that the more cells there are, the more formazan product will form from MTS tetrazolium salt. This project can be divided into three parts (each experiment was done with two media condition – medium only and CDDP-treated media):

Evaluating endogenous CS/DS effects towards CDDP chemosensitivity

1. Silencing of CHSY1 gene responsible for CS/DS biosynthesis and degrading endogenous CS/DS with Chondroitinase ABC (ChABC) were carried out to determine endogenous CS/DS effect.  
2. CDDP effect towards endogenous CS/DS amount is obtained.  
3. Endogenous CS/DS sulfation sites were evaluated of their role in cell proliferation in CDDP presence through sodium chlorate treatment which prevents sulfation in GAGs.

Evaluating exogenous CS effects towards CDDP chemosensitivity

Exogenous CSA, CSC, CSD, CSE and CSO were given during treatment period with CDDP.

Evaluating DS effects towards CDDP chemosensitivity

Exogenous DS was added during treatment period with CDDP. A follow-up experiment was carried out with Chondroitinase B (ChB) to evaluate endogenous DS effect towards CDDP.

RESULTS AND DISCUSSION

Evaluating endogenous CS/DS effects towards CDDP chemosensitivity

Silencing CHSY-1 Gene Expression Significant decreases in cell growth were observed in both medium only and CDDP-treated media. The reduction of CS/DS biosynthesis could have caused a reduction of chondroitin sulfate proteoglycans (CSPGs) which are substrates for matrix metalloproteinases (MMPs) that play a role in cancer cell survival (Niina, 2007). In addition, tissue inhibitors of metalloproteinases (TIMPs), a potential tumor suppressor (Jiang, 2002), could be overexpressed in the presence of CDDP, hence decreasing cell surviving rate.

Degradation Endogenous CSA, CSC and DS with ChABC After treatment with ChABC responsible for degrading CSA, CSC and DS, significant decrease in cell growth was observed in the CDDP-treated media. This could suggest that endogenous CSA, CSC and DS play a role in their own individual ways, together as a complex or aid one another in enhancing cell proliferation in CDDP presence. CS/DS can function as a metal ion chelator and reduces molecular damage. These GAGs could have inhibited CDDP by chelating Pt(II) (Campo, 2006) before CDDP gets a chance to denature the DNA double helix and kill the cells.

Evaluating CDDP Effects towards Endogenous CS/DS Amount After normalizing the results, CDDP is observed to have a significant effect on endogenous CS/DS decreasing their amount
and hence decreasing the proliferation potential. We could postulate that CDDP is an effective chemotherapy drug against these GAGs in metastatic MDA-MB-231 cells by adding ChABC.

**Evaluating the Importance of CS/DS Sulfation Sites**

Both media conditions displayed significant decreases in cell proliferation. This results supports previous experiments that undersulfation of GAGs reduces proliferative activity of cells. (Humphries and Silbert, 1988) Hence, we could conclude that CS/DS sulfation sites do play an important role in cell growth in CDDP-treated media.

**Evaluating Exogenous CS Effects towards CDDP Chemosensitivity**

Different CSs gave different cell growth level. Significant increase was observed in CSA and CSO treated medium only condition. Only CSE gave significant increase in the CDDP-treated media.

**Number of sulfation sites**

It is possible that the number of sulfation sites could have affected the CS interaction with CDDP and GAG cell signaling. The strength of binding GAGs to various molecules is primarily determined by the degree of sulfation of the GAG. (Ruoslahti, 1989) CSE having two sulfated sites is able to initiate an increase in cell proliferation in CDDP presence till a concentration of 100ng/ml unlike the single-sulfated CS type (CSA and CSC).

**Sites of sulfation**

The position of the sulfated sites could play a role in the cell proliferation level. CSE has sulfation sites at Carbon 4 and 6 of the GalNAc while CSD’s sulfation sites are at Carbon 2 of GlcA and Carbon 6 of GalNAc. CSs with low amount of 2,6-disulfated disaccharide and low charge density strongly stimulate proliferative potential. (Volpi, 1993) This may be part of the reason why CSE and not CSD improved cell proliferation level though both have two sulfated sites each.

**Evaluating DS Effects towards CDDP Chemosensitivity**

**Exogenous DS Effects**

A linear trend of negative gradient and significant decreases were interestingly observed in the CDDP-treated group. This could suggest that DS actually aids CDDP in killing cells i.e. the higher is the concentration of exogenous DS in CDDP presence, the higher is the toxicity level. This could be due to its number of sulfation sites and position of the sites. DS, a flexible GAG, can have one to three sulfation sites. In addition, the composition of DS consisting of IdoA instead of GlcA could also be a key player in its biological activity.

**Degradation of Endogenous DS**

In the ChB and CDDP treated cells, a significant increase was observed, indicating with the absence of DS, cell proliferation is higher in CDDP presence. This re-confirms that DS can cooperate with its killing partner, CDDP in lowering cell proliferation. These findings can also indicate that chondroitinases (like ChB) may not always be useful as therapeutic agents to prevent or inhibit tumor growth and metastasis. (Denholm, 2001)

DS chain binding affinity to molecules is changeable under different physiological and pathological conditions. This is due to its structural heterogeneity connected with differential degrees of monosaccharide residue sulfation and GlcA epimerization. The latter modification determines DS chain flexibility, hence influencing the GAG bio-reactivity. (Casu, 1993) In addition, it has been reported that high amounts of IdoA can inhibit proliferation of normal
fibroblasts than those with GlcA. DS in CDDP media may also have adopted this mechanism, hence inhibiting cell growth. (Westergren-Thorsson, 1991)

CONCLUSION

Different CS/DS have different effects towards CDDP chemosensitivity. A balance of specific CS/DS type is hence important in CDDP presence. It is proposed that further studies need to be done on CS/DS and CDDP effects at the molecular level to understand their mechanism and improve metastatic breast cancer treatments.

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