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Computer-Aided Approach to Study the Anti-Tumour Potentials of Seaweed against Canine Breast Cancer

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Abstract—*Among all the type of cancer, the incidence of breast cancer in canines has been increasing despite the fact that the efforts to prevent the disease has been improving. The traditional chemotherapy has been shown to be effective though it exhibit some serious adverse effects as the targets of the chemotherapeutic agents are nonspecific in most cases. Hence the chemotherapeutic agents affect the normal cells leading to the adverse effects. However researchers have been working for the past two decades to find the alternative treatment to the cancer with lesser adverse effect. Natural products or the bioactive compounds from natural sources have been proved to be less toxic. Fucoidan is one such natural polysaccharide of sulphated fucose residues from seaweed. Fucoidan have been used in cancer treatment in many countries. In this study we analyse the ability of fucoidan to treat breast cancer using in silico approaches.*

Keywods*: Breast cancer – anti-cancer effect -sea weed - in-silico study.*

1. INTRODUCTION:

Cancers are multifactorial diseases which arise largely as a consequence of acquired genetic mutations that alter cell function leading to neoplastic cells to survival or growth advantages [7]. Cancer causes death mostly through metastatis, which is the spread of tumour cells to distal organs. Unbalance programmed cell death, disordered signalling pathways, angiogenesis (generation of new blood vessels) and poor immune response against cancer lead to the disruption of various pathways in tumour development [2].Breast cancer among all the types of cancer, has been a major concern as the incidence of breast cancer increases though the preventive measures had been increasing. Chemotherapy has been a major treatment for cancer for a period of 60 years. Different chemicals ranging from traditional agents such as methotrexate to synthetic chemicals have been used in chemotherapy [9]. In spite of promising tumour growth inhibitory effects in pre-clinical trial, many of them fail in clinical trial which considers the adverse effects. Normally chemotherapeutic agents targets the cells those proliferate in higher rate, assuming those are the cancer cells. So normal cells, with rapidly dividing capacity also affected during chemotherapy. Recently novel therapeutic agents which targets specific molecules have been designed and used only to found out that they are also not completely free of adverse effect [4]. Moreover chemotherapy causes tumour cell resistance and development of secondary cancers from chemotherapeutic chemicals used. Hence the cancer research focuses on the natural products instead of harmful chemicals [6].

There are multiple types of mammary tumors in dogs. Approximately 50% of all mammary tumors in dogs are benign, and the other 50% are malignant. The most common benign form of canine mammary tumors is actually a mixture of several different types of cells. For a single tumor to possess more than one kind of cancerous cell is actually rare in many species. This combination cancer in the dog is called a 'benign mixed mammary tumor' and contains glandular and connective tissue. Other benign tumors include complex adenomas, fibroadenomas, duct papillomas, and simple adenomas.

Prompt surgical removal of any mammary tumor is recommended, unless the dog is very old or has other medical conditions that would rule out this option. If a surgery is in the early stages of this disease, the cancer can be totally eliminated in over 50% of the cases having a malignant form of cancer. Sometimes, only the mass itself will be removed. This is usually only done when the tumor is very localized. However, because of how this cancer spreads, it is generally advisable that radical surgery be performed which entails the

removal of the mass and all the mammary tissue and lymph nodes that drain with the gland. With some types of tumors, especially sarcomas, complete removal can be difficult and many of these cases will have tumor regrowth at the site of the previously removed tumor. Radiation therapy, chemotherapy and anti-estrogen therapy alone have been used to treat inoperable mammary cancers, although not very successfully.

In advanced mammary cancers where metastasis has occurred or in which the tumor is fixed to the underlying tissues, surgery will not be curative but may be considered an option to reduce local tumor-burden and improve quality of life.

Owners may confuse the surgical removal of a mammary gland of a dog with a radical mastectomy in humans. In humans, this type of surgery would affect the underlying muscle tissue, which complicates the recovery. In dogs, all of the breast tissue and the related lymphatics are outside of the muscle layer, so it is necessary to only to cut through the skin and the mammary tissue. This makes the surgery much easier and recovery much faster. A radical mastectomy in dogs means all the breasts, the skin covering them, and the four lymph nodes are all removed at the same time. Although this is major surgery, normal activity will likely resume after the sutures have been removed (10-14 days post surgery).

Many veterinarians will spay a dog having a mastectomy. There is evidence that she will benefit by having the ovaries and uterus removed because it can reduce her chances of masking her tumors with a false pregnancy and eliminates the risk of ovarian and uterine tumors. Additionally, spaying will allow easy detection of any new tumors that may arise because following surgery, the mammary tissue will shrink (atrophy).

In veterinary hospitals with the necessary equipment, sometimes surgery will be performed with intraoperative radiation in an effort to reduce the chances of local recurrence. Follow up radiation therapy to the primary site and draining lymph node may also help reduce the problem of local recurrence and local metastasis.

The most commonly used chemotherapy protocol for the prevention of metastases from malignant breast cancer in dogs had been Adriamycin every 21 days and oral Cytoxan every other day for 8 weeks or on day 3-6 of each 21 day cycle. However more recently, many oncologists have switched to the use mitoxantron (Novantrone™) as a first choice and then Adriamycin or Carboplatin for resistant disease. Treatment with chemotherapy may reduce the ability of the circulating cancer cells to metastasize to the lungs.

Some veterinarians recommend supplements that are shown to reduce the risk of breast cancer for women: Inositol hexaphosphate (IP6 derived from rice), and 1-3-beta glucan (derived from yeast). It can make sense to recommend these products for life since intact female dogs have the highest incidence of breast tumors than any other companion animal and three times the incidence of breast tumors than women. If these "Chemoprevention supplements are added to the diet, they may play a role in the prevention of recurrent breast tumors in predisposed dogs.

Clinical studies examining the efficacy of the systemic chemotherapeutic agent Tamoxifen (a drug often given to women with breast cancer) for advanced mammary cancer in dogs has shown no measurable therapeutic gain in any of the dogs within the study.

Fucoidan is a polymer of sulphated fucose residues whose richest source is marine algae species such as *Laminaria*and *Fucus*[13]. Fucoidan is preferred in therapy in humansdue to its low toxicity, oral bioavailability, multiple mechanism of action and the simple extraction process. Fucoidan affects many pathophysiological processes, including inflammation, carcinogenesis, vascular physiology and oxidative stress [5]. Fucoidan containing food supplements have been administered to cancer patients in Japan, Korea, China and other countries. Fucoidan can directly induce cytotoxicity and apoptosis [15] and indirectly acts as a antiangiogenic agent and has immune-stimulating effects on dentric cells (DCs) [10, 17, 8, 12]and natural killer (NK) cells [1,3].

In recent years, Computer-aided drug design has been a major breakthrough for discovering lead molecules and to find the relationship between the structure and the activity of small molecules. Molecular docking study predicts the characteristics of the binding of the small molecules with the receptor or the target [11]. In this study we performed molecular docking of various targets for breast cancer in canines with the polysaccharide fucoidan. The name of the target and their role in cancer has been listed out in table 1.

2. MATERIALS AND METHODS:

The 3D structures of the target proteins (breast cancer) of canine were retrieved from the Protein Data Bank (PDB) [14]. The heteroatoms such as the water molecules and the ligands were removed in Accelry's Discovery Studio 4.0. CHARMm forcefield was applied to the protein before finding the possible binding sites from receptor cavities. The 3D structure of the ligand, fucoidan was retrieved from the PubChem database [16]. The ligand geometry was cleaned to perform flexible ligand docking. LIGANDFIT which performs docking based on the cavity detection algorithm was the docking method used for our study (Accelyr's Discovery Studio 4.0) and allow us to virtually screen compounds and predict the strongest binders based on various scoring function. For our study the molecular docking analysis of AP-1 with ligands was carried out using Dreiding parameter in which the partial charges of target protein and ligand in which the Gasteiger charging method was employed to calculate. The energy grid extension was set to 5.0A° and '0' was set as the conformation search number of Monte carlo trial. The number of poses for ligands in receptor cavity was limited to 10 and other input parameters for docking were set as default options and docking was performed. BroydenFlecher Gold Farbshanno

(BFGS) methods is employed on LIGANDFIT for the final energy refinement of the ligand pose (or) pose optimization.

3. RESULT AND DISCUSSION:

The targets were identified from previously published research articles. The name, role in breast cancer and the PDB ID of the targets are tabulated in the following table.

Table 1 – List of Targets

Target	Role in breast cancer	PDB ID
BCAR3	Estrogen-independent proliferation of breast cancer cells	3T6A
BRCA1	Maintain genome stability and acts as a tumour suppressor	1JM7
Caspase 3	Apoptosis	4QTX
BRMS1	Reduces the metastatic potential	4AUV
ER α	implicated in pathological processes including breast cancer	4JC3
IGF-1R	anti-apoptotic agent	5HZN
MMP9	tumour invasion	2OVX
MMP3	tumour initiation	1QIA
PCNA	Involved in cell cycle	4ZTD
TGFBR	Frequently upregulated in tumour cells	3KFD
Cathedepsin D	increases the metastasis potential	4OBZ
MMP12	Involved in metastasis	2N8R
PPARG	Implicated in the pathology of cancer	1WM0
Tenascin	Metastasis	2RB8
Twist	Tumour denelopment	2MJV
ER β	Tumour development and progression	2YLY
ERBB4	Mitogenesis and differentiation	2AHX

Docking was performed between the various targets and the fucoidan residue. Dockscore is a scoring function that depends on many parameters of the interaction. Higher the dockscore, better the interaction or lower the binding energy. The poses which had the maximum dockscore have been selected for further processing. If the ligand has bound with more than one binding site, the final pose which had the highest dockscore among those sites was selected as the ligand binds at the site which requires minimum energy for binding. As a dockscore of 40 is considered good or better interaction, the interactions with a dockscore less than 40 were neglected. The number of binding sites, the site at which the best interaction has occurred and their corresponding dockscore have been listed out in the following table.

Table 2 – Scoring functions

Protein	Dock score	Site	Total binding sites
ER alpha	311.432	2	5
MMP3	228.99	14	18
BCAR 3	61.126	4	22
ER beta	58.817	5	16
IGF 1R	54.465	4	20
Aromatase	53.998	3	13
TGFBR	51.998	2	39
PCNA	51.356	11	29
Estronesulfatse	48.954	12	17
Er Bb-4	48.654	14	16
BRMS	41.071	5	7
MMP9	40.35	6	7

The interacting amino acids of the proteins with the fucoidan were found using view interaction tool in Discovery studio and the 2D diagram of the interactions are shown below.

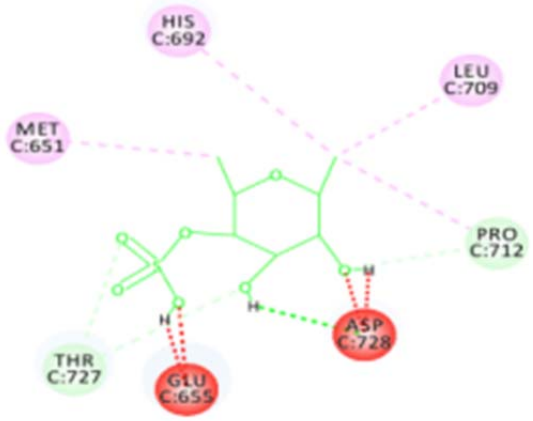

Fig. 1 – Interaction of BRCA3

Fig. 2 – Interaction of TGFBR

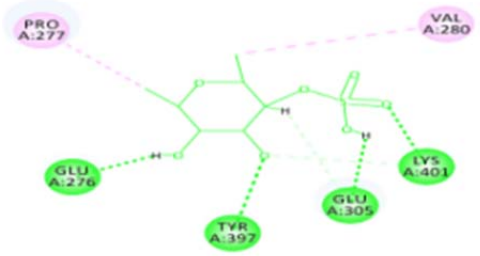

Fig. 3 – Interaction of ER β

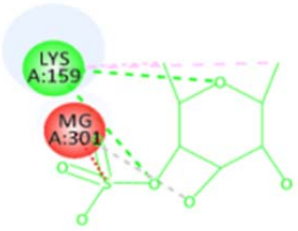

Fig. 4 – Interaction of ER α

The red, pink, light and dark green colours bonding indicate unfavourable bump, alkyl, carbon hydrogen bond and conventional hydrogen bond respectively. The presence of unfavourable bump does not affect the interaction if it has higher dock score as the dock score is calculated based on all of these interactions. The number of hydrogen bond also plays a critical role in determining the strength of the interaction. More the number of hydrogen bond of lesser bond length results in better binding. The ligand has been illustrated in green colour. The interacting amino acids are labelled with 3 letter code and their position in the peptide chain with the colour indicating the type of bond it forms with the ligand i.e., fucoidan.

The above results show that the sulphated polysaccharide has wide range of target for breast cancer including metastasis and proliferation. Thus it can be used as an alternative for chemotherapy, since it has lesser toxicity than that of the chemotherapeutic agents. The availability and the simple extraction procedure also make the preparation easy. Further *in vivo* and *in vitr*o experiments has to be performed for analysing the efficacy of fucoidan in the treatment of breast cancer in canines.

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