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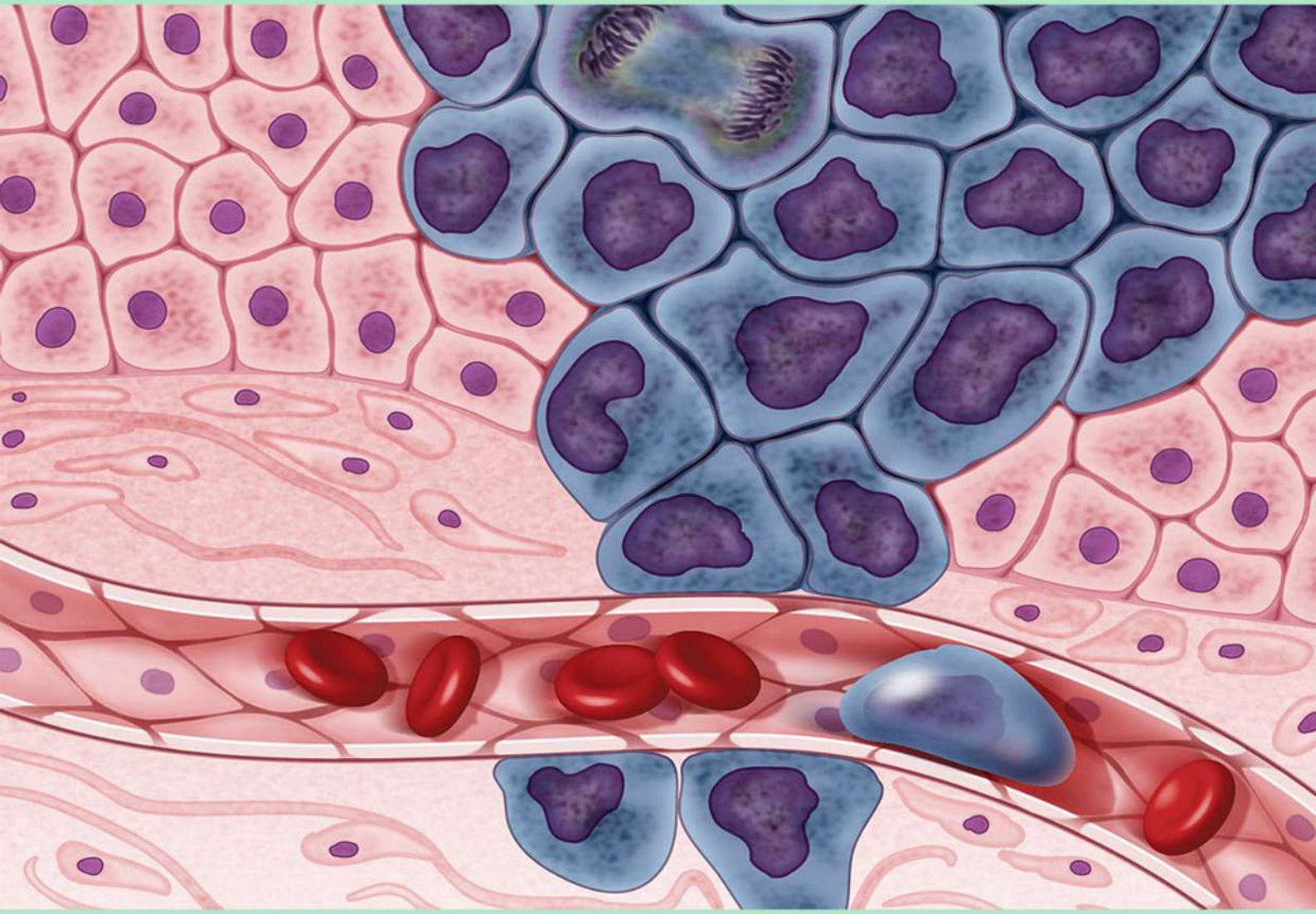

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Editorial

Engaging African Americans in Breast Cancer Prevention Strategies: A Partnership Between a Community Cancer Center and the African American Community in Delaware

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Although breast cancer incidence has been stable for non-hispanic whites since the early 2000s, incidence rates of invasive breast cancers in African Americans (AA) are rising (0.9%/year 2005-2014).[1] This is especially true for younger black women. Breast cancer incidence in black women under 45 years has recently surpassed that of their white counterparts.[2] This is concerning as breast cancer specific mortality is higher in African Americans than all other races. Although many breast cancer risk factors have been identified, there is a need to develop breast prevention strategies specifically targeting the AA community.

A potential factor contributing to increased breast cancer incidence in African Americans is an increasing obesity rate among this population. A recent study using ecological analysis to quantify the long-term effect of breast cancer risk factors found that black women younger than 40 years experienced the largest increase in mean body mass index (BMI)compared to white women over the same period. Likewise, African Americans have higher BMI at breast cancer diagnosis and this may be related to presentation with later stage disease.[3] Guidelines developed by the American Cancer Society (ACS) and World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) to aid in cancer prevention efforts include maintaining a healthy body weight, increasing physical activity, eating a nutritious diet, avoiding smoking and reducing alcohol consumption may be helpful to mitigate increasing breast cancer incidence in African Americans. However, a recent study in a prospective cohort of 566,398 adults showed that adherence to these guidelines is lowest among the African American population. In this study, adherence to one or less of the risk factor guidelines had a 38% increased risk of breast cancer (HR: 1.38, 95% CI: 1.25-1.52).[4]

In addition to decreasing breast cancer risk through modifiable lifestyle factors, genetic counseling and testing can substantially lower breast cancer risk by identifying women who carry hereditary mutations.[5] Testing results can be used to recommend preventative interventions and enhanced screening options for these patients, and can also enable risk assessment among relatives who may also benefit. Furthermore, participation in genetic counseling has been shown to increase the accuracy of risk perception, reducing cancer related anxiety and depression.[5] Although AA have the same risk for hereditary breast cancer as Caucasians, their participation in genetic counseling and testing is substantially lower.[6] A recent meta-analysis of studies investigating access, perceptions, knowledge and attitudes about genetic counseling reported low awareness and knowledge of genetic counseling/testing by minority groups including African Americans. The authors recommended "to increase awareness and knowledge of genetic testing for cancer risk and to reduce the perceived stigma and taboo surrounding the topic of cancer in ethnic minority groups".[7]

As one of the first community cancer centers in the country to have a genetic counseling program integrated within our multidisciplinary centers and clinical care, the Helen F Graham Cancer Center and Research Institute (HFGCCRI) has proven success with identification and referral of early onset and triple negative breast cancer cases. However, analyses of African American breast cancer patients undergoing genetic counseling at our institution parallel the national trends. Data from the HFGCCRI

cancer registry indicates that although African Americans make up 21% of patients diagnosed with early-onset breast cancer, only 15% of eligible African American patients had undergone genetic counseling. This disparity is further increased for African American women with triple negative breast cancer, which is more prevalent in the African American population. All women who are diagnosed with triple-negative breast cancer (TNBC) at age≤60 years old are automatically eligible for genetic counseling, regardless of family history. Although African American women represent 41% of this population, their participation in genetic counseling remains around 15%. This is particularly concerning as the state of Delaware ranks among the highest in incidence of triple negative breast cancer. Likewise, consistent with national numbers, breast cancer incidence is rising among African Americans in Delaware.[8]

To address these breast cancer disparities, the Breast Cancer Research Program at the HFGCCRI formed a Community Research Advisory Board (CRAB) composed of stakeholders in the Delaware breast cancer community including members of local African American sororities. Membership in the CRAB required the commitment of each member to: (1) contribute to HFGCCRI community based participatory research (CBPR) projects related to breast cancer (2) promote dialog between community members, breast cancer clinicians and researchers (3) serve as liaisons to establish trust and understanding of research activities in the community. (4) provide and promote education and dissemination of health, prevention and research information to the community.

Although much research has been done into causes of health disparities in breast cancer, dissemination of findings and implementation of strategies to overcome these disparities has been slow. In conversations with our CRAB, several critical points regarding the AA community were brought to our attention: (1) There is a lack of understanding in the AA community about different types of breast cancer and how treatments and outcomes may be different depending on the breast cancer subtype. (2) There is lack of knowledge about genetic counseling and the benefits of genetic testing for breast cancer prevention (3) There is lack of knowledge of general breast cancer prevention strategies. For example, members of the community were not aware of the linkage between obesity and breast cancer risks or the fact that breastfeeding can reduce risk of breast cancer and (4) Although trust issues remain one of the driving factors for lack of participation by African Americans in research, members also felt that there is a lack of appreciation of the benefits participation in clinical trials and genetic research by African Americans. Given this information, it was the consensus of the board that the most immediate community need was one of education and that our first project would be a community educational program to address these specific gaps in knowledge.

Using a community-based participatory research (CBPR) model, the CRAB choose a peer education platform (Train the Trainer) for delivery of the educational program. In contrast to a traditional medical format for breast cancer education, the community members of our board recommended that the presentation be in a story telling format. "The Story of BRENDA™" is presented from the viewpoint of a young AA woman who has recently been diagnosed with triple negative breast cancer.[9] Throughout the presentation, BRENDA leads the audience through a series of her questions related to triple negative breast cancer, genetic testing, participation in research and breast cancer prevention. The trained community moderator then provides the information to answer each of these questions utilizing a script written by members of the board as talking points. At the end of the presentation, the audience is asked to remember the name BRENDA which is an acronym for the WCRF/AICR guidelines for breast cancer prevention: Breastfeed, Reduce consumption of alcoholic and sugary beverages, Exercise, Nutritious foods, Do not smoke and Achieve a healthy body weight. Attendees are also provided with a tool-kit containing more information about breast cancer and resources to determine if genetic counseling may be appropriate. Contact information to a genetics counselor and/or navigator for mammography is also distributed. The number of referrals for genetic counseling and navigation resulting from this education are tracked and used as an outcome measure. To allow for monitoring of the programs outcomes, as well as collection of data about community attitudes and beliefs about breast cancer, participants are given a short survey directly following the program.

As we have just completed the pilot phase of this program, it is too early to determine its success in creating a sustainable community education platform for the AA community and what impact it will have on breast cancer disparities within the state long-term. To increase sustainability, the program has been integrated into the cancer center's outreach department, which conducts the educator training, coordinates event scheduling for community members sponsoring the presentation and serves as a liaison for program participants who seek genetic and/or navigation services at the cancer center. Although still in its early stages, the Story of BRENDA™ has been well received by the community and several participants have sought genetic counseling. As we move to the next phase of the project, we hope to strengthen our partnership with the AA community and as we build on this program to reduce breast cancer incidence and improve outcomes for all women with breast cancer in the state.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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Original Research

Deep-Ultraviolet Raman Spectroscopy for Cancer Diagnostics: A Feasibility Study with Cell Lines and Tissues

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ABSTRACT

Background
Deep-ultraviolet resonance Raman spectroscopy (UVRS) offers significant advantages over visible and near-infrared Raman spectroscopy for biological applications, including cancer identification. Cancer is the second-leading cause of death in the United States. Early diagnostics plays a crucial role in providing the best chances for an afflicted individual to seek successful treatment opportunities. Current methods for diagnosing various forms of cancer are both expensive and invasive. As such, the objective of this study is to explore the feasibility of UVRS for discrimination of cancerous tissues and cancer cells from normal samples. The safety issues of using ultraviolet light for human applications are analyzed.

Methods
Cancerous brain tissues from nonobese diabetic/severe combined immunodeficiency (NOD-SCID) model mice injected with 435-tdT cells (human adenocarcinoma breast cancer cells) at known locations and adjacent normal brain tissues as well as normal and cancer (adenocarcinoma PC-3) prostate cells were studied using UVRS. The obtained Raman spectra of the healthy and cancerous samples are compared in order to identify biochemical differences between them.

Results
The obtained spectra reflect biochemical differences which occur between the healthy and malignant samples in both brain and prostate cancers. UVRS provides distinctive resonance signatures of major biochemical components, including proteins and nucleic acids, and it does not suffer from fluorescence interference, nor does it require high laser power levels for excitation. These advantages allow for clear and effective spectral discrimination between samples.

Conclusion
Our results suggest UVRS should be considered for cancer identification, and is safe for use within humans. The proposed innovative approach has significant potential for cancer imaging and real-time tissue discrimination during surgery.

Keywords
Cancer diagnostics; Deep-ultraviolet resonance Raman spectroscopy; Prostate cancer; Brain cancer; Cancer imaging systems.

Abbreviations
UVRS: Deep-ultraviolet resonance Raman spectroscopy; RS: Raman spectroscopy; CARS: Coherent Anti-Stokes Raman Scattering; SRS: Stimulated Raman Scattering; UV: Ultraviolet; IR: Infrared.

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INTRODUCTION

Cancer is known as one of the most dreaded diagnoses a doctor can make. The time, pain, effort, and oftentimes heartache associated with a cancer diagnosis will undoubtedly impact most people, either directly or indirectly, at least once in their lifetime. Cancer is the second-leading cause of death in the United States, and in 2018 alone, 18.1 million new cases of cancer were identified.[1]

Current methods for diagnosing cancers generally include lab tests conducted on body fluids, imaging exams, and biopsies. These tests are time-consuming and invasive for the patient. Specific and accurate diagnoses are not always made, with the potential for misdiagnoses to occur. There remains a need for the development of a much more accurate and sensitive method which can universally diagnose cancer and could be used in cancer imaging systems during surgery.

Raman Spectroscopy (RS) is a powerful analytical technique that allows detection and measurement of basic molecular classes in complex biological samples, such as body fluids, cells, and tissues.[2-4] Therefore, the RS technique has a great potential for use as a diagnostic tool.[5,6] RS identifies chemical specificity of molecular species without staining or labeling. The RS spectral "signature" may be used to build multivariate calibration and classification models for practical clinical needs.[7,8] Raman signals from tissues originate from laser light backscattered by molecules. This allows for the collection of the backscattered light into the same optical fiber used for tissue illumination, which makes RS a practical tool for *in vivo* diagnostics and for examination of tissue specimens. RS system for tissue diagnostics can readily incorporate recent advances in the design of optical light collectors, miniaturization of lasers, and noise reduction in photon counting devices (e.g., cryogenically cooled charged coupled device (CCD) cameras). It is able to complement other techniques and, in many cases, provide new information unattainable by other approaches.[9-11] The ability of RS to recognize and quantify biological molecules (lipids, proteins, DNA, etc.) through their unique vibrational signatures has been well demonstrated.[12-19] Traditional RS has two main drawbacks: (1) a low efficiency (one RS photon is detected per 107 photons irradiating a sample) of the inelastic light scattering compared to elastic scattering[20,21] and (2) inherent fluorescence which accompanies RS studies.[22] For example, using a traditional 785 nm excitation for RS, one typically records low-level noisy Raman signals. Acquisition of a high-quality spectrum requires extended accumulation time with cryogenically cooled CCD cameras, which limits the application of RS for tissue imaging involving hundreds or thousands of spectral measurements.

Coherent anti-stokes Raman scattering (CARS) can improve the efficiency of RS.[23] In CARS, coherent vibrations of the molecules are driven at a selected frequency by simultaneous action of "pump" and "Stokes" lasers. The wavelength of a laser is fixed and that of another laser is tuned over a narrow range to excite the molecular vibrational mode of interest.[24-26] The major drawback of CARS is the existence of a non-resonant background.[27] Additionally, the CARS signal is decreased quadratically with the molecular concentration, making detection of low-abundance molecular species difficult. CARS requires the use of two rather expensive lasers generating picosecond pulses.[28] Also, due to a nonlinear physical process with ultra short laser pulses, a broadening of recorded Raman bands is observed, which can be detrimental to chemical specificity.[29] Because the signal generation in CARS critically depends on the tight overlap of the pump and Stokes laser foci, keeping this overlap constant can be problematic within the *in vivo* environment.[30]

Stimulated Raman Scattering (SRS) also utilizes "pump" and "Stokes" lasers to produce trains of picosecond pulses. When the difference between the frequencies of the "pump" and "Stokes" photons match the frequency of a molecular vibration, stimulated emission occurs; thus, the intensity of the pump laser transmitted by the specimen decreases while that of the Stokes beam increases. High-frequency modulation is used.[31,32] Though SRS showed a great promise in studying thin tissue slices and cell cultures,[33] its application to *in vivo* diagnostics has technical difficulties. The problem of keeping a tight overlap between the foci of two laser beams is also pertinent to SRS. Due to its nonlinear nature, the SRS signal is affected by variations in tissue composition (local density, presence of lipid droplets), non-Raman background, and photo damage.[34]

Deep-ultraviolet resonance Raman spectroscopy (UVRS) may be used as an alternative method for detecting cancer within biological specimens. It has been shown that a low RS yield can be dramatically increased by using excitation wavelengths near molecular electronic transitions (resonances).[35] The strength of Raman signals, caused by excitation of vibrational modes associated with that transition, exhibits an exponential increase.[36] The inelastic scattering by biological samples is increased many-fold around 200 nm due to strong electronic absorption of amide groups.[37] Accordingly, the use of ultraviolet (UV) excitation can cause a thousand-fold increase in the Raman signal because the Raman cross section increases by the 4th power of the excitation light frequency.[37] This makes UV resonance RS indispensable for studying protein structure and transformation,[38-40] understanding fibrils and fibrillogenesis,[41-44] analyzing bacteria and microorganisms,[45-47] and various other biological applications. Most importantly, the use of laser wavelengths below 250 nm eliminates the problem of autofluorescence which impedes Raman studies on tissues using near-infrared lasers.[48-50] Contrary to CARS and SRS, the UV resonance RS may simultaneously provide polychromatic information about Raman signatures of the tissue or body fluid being analyzed. An advanced processing of Raman spectra considerably improves tissue identification.[51] Overall, UV excitation allows significant reduction of irradiation power to address safety concerns and to generate high signal-to-noise ratios for Raman spectra, as well as decrease its accumulation time. These factors are of great importance for a new UVRS technology.

The objective of this study is to explore the feasibility for deep-ultraviolet resonance Raman spectroscopy to be used for cancer detection in prostate cancer cell lines and cancerous

brain tissues. Results show that UVRS is able to identify biochemical differences between malignant and healthy biological samples, thus opening the door for future studies to occur using UVRS for cancer imaging during surgery.

MATERIALS AND METHODS

Human Prostate Cell Culture

Normal human primary prostate epithelial cells (HPrEC) (ATCC® PCS-440-010™) and grade IV adenocarcinoma PC-3 prostate cancer cells (ATCC® CRL-1435™) were used for this study. The cells were obtained from the American Type Culture Collection (ATCC).

The HPrEC cells were cultured in 75 cm^2 tissue culture flask (Corning® T-75 flasks catalog #430641) using Complete Growth Medium (ATCC). To make the complete growth medium, L-Glutamine, Extract P, Epinephrine, rhTGF-a, Hydrocortisone hemisuccinate, rh Insulin and Apo-transferrin at a final concentration of 6 mM, 0.4%, 1.0 mM, 0.5 ng/mL, 100 ng/mL, 5 mg/mL, and 5 mg/mL respectively were added to the Prostate Epithelial Cell Basal Medium (ATCC® PCS-440-030™). The HPrEC cells were passaged when the cells reached approximately 80% confluency. The flask was rinsed twice with 5 mL D-PBS (ATCC 30-2200), and 5 mL pre-warmed trypsin-EDTA solution (ATCC® PCS-999-003) was added and incubated at 37 °C CO_2 incubator for 1-2 minutes. The flask was then gently tapped from all sides to facilitate detachment of the cells from the surface of the flask. An equal volume of the Trypsin Neutralizing Solution (ATCC PCS-999-004) was added to the flask and the cells were collected in a 15 mL tube. The tube containing the trypsin-EDTA-dissociated cells was centrifuged at 1000 rpm for 5 minutes. The solution was then aspirated from the cell pellet and the cells were resuspended in 10 mL of pre-warmed complete growth medium, at which point the cell numbers were counted. The cells were then seeded in a new tissue culture flask at a density of 5,000-6,000 viable cells per cm^2 and grown at 37 °C in a 5% CO_2 incubator for 2-3 days until they reached 80% confluency.

The PC-3 prostate cancer cells were cultured using Complete Growth Medium (ATCC). The base medium for this cell line is F-12K Medium (ATCC Catalog No. 30-2004). To make the complete growth medium, fetal bovine serum (ATCC) was added to this medium at a final concentration of 10%. The cells were cultured using 75 cm^2 tissue culture flask and subcultured following the similar procedures as described above for HPrEC cells.

Mouse Brain Tissues

The cancerous brain tissues were obtained from NOD-SCID-model mice injected with 435-tdT cells (human breast adenocarcinoma cancer cell line, MDA-MB-435)[52,53] were provided by Prof. V. Raman, Johns Hopkins University School of Medicine, (Baltimore, MD, USA).[54] The adjacent normal brain tissues were obtained for comparison. The characterization of such cancerous and normal brain tissue sections was carried out in an earlier study.[55]

Sample Preparation

The prostate cancer and healthy cell lines were received and analyzed immediately following cell culturing. For analysis, the cells were dispersed in about 150 μL of water.

The mice brain tissue was removed from the glass slide and separated into two tubes. One tube contained tissue from the identified normal tissue area and the other tube contained the cancerous tissue; the tissue cancer mapping was provided.[54] The tissue was mixed with 150 μL of water and sonicated to create a water dispersion.

Deep-UV Spectroscopic Methods

All samples were analyzed using a deep-UV Raman Spectrograph (details regarding instrument can be found elsewhere).[56] Briefly, 198-nm radiation was generated as the 5th anti-Stokes shift from the third harmonic of the Ni-YAG laser in a Raman shifter filled with low pressure hydrogen. A UV laser beam (~0.5 mW at the sample surface) was focused into a spinning Suprasil nuclear magnetic resonance (NMR) tube containing the solution. In order to prevent photo degradation, the solution was continuously mixed with a magnetic stirrer. Scattered radiation was collected in the backscattering geometry, dispersed using a double monochromator, and detected with a liquid-nitrogen cooled CCD camera (Roper Scientific, New Jersey, USA).

Data Acquisition and Treatment

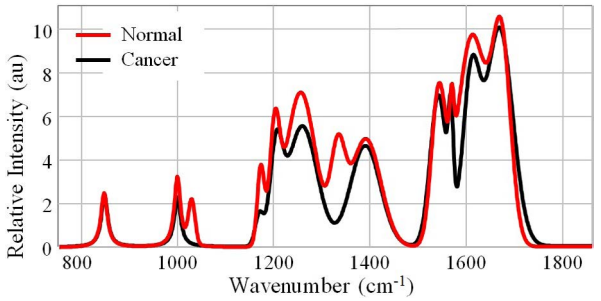
For the collection of data from the cell samples, the accumulation time was 30 seconds, and 20 acquisitions were obtained and averaged for each spectrum. For the tissue samples, the accumulation time remained the same, however, 40 acquisitions were collected and averaged for each spectrum. The collection of multiple acquisitions per sample is conducted in order to account for the inherent heterogeneity of biological samples. The contribution of quartz from the NMR tube and of water was quantitatively subtracted from each spectrum. The spectra were preprocessed using GRAMS (version 9.2) software. Spectra were calibrated to wave numbers, using the Raman spectrum of Teflon as a reference, and smoothed.

RESULTS

Deep-UV resonance Raman spectra were collected from two different biological sample types. Tentative assignments were given to those bands observed in the Raman spectra using deep-UV excitation based on assignments from the literature which were obtained using near-IR and visible excitation.

First, prostate cancer and healthy cell lines were analyzed. The mean spectra for each of the two cell lines arepresent in Figure 1.

Figure 1. *Average Spectra Collected from Water Solutions of Normal Prostate Cells (Red) and Cancer Prostate Cells (Black) of a Human Using Deep-UV Raman Scattering*



The spectra show general differences in protein structure and conformation, noted by variances in peak intensities at 1000, 1257, 1542, 1614, and 1669 cm^{-1}.[57,58] Collagen can tentatively be assigned as contributing to the Raman spectra at 1173, 1206, and 1334 cm^{-1}.[11,57,59] Various vibrational modes of DNA are observed at 1173, 1206, 1257, 1568, and 1614 cm^{-1} and by lipids at 1381 cm^{-1}.[60-62] Glycogen most likely contributes to the Raman spectrum as observed by the Raman bands at 850 and 1030 cm^{-1}.[57,63] A full list of tentative assignments for the vibrational modes of the various Raman bands is shown in Table 1.

Table 1. *Tentative Raman Shift Assignments of Spectra Collected From normal and Prostate Cancer Cell Lines Using UVRS*

Shift (cm^{-1})	Assignment	Contribution
850		Glycogen
1000	Phenylalanine symmetric ring breathing	Protein
1030		Glycogen
1173	Tyrosine, Cytosine; Guanine	Collagen; DNA
1206	Hydroxyproline, Tyrosine	Collagen; DNA
1257	Adenine and Thymine ring breathing; Amide III	DNA; Protein
1334	CH_3CH_2 Wagging	Collagen; Polynucleotide Chain
1381	CH_3 symmetric stretching	Lipids
1542	Amide II	Protein
1568	Guanine and Adenine ring breathing	DNA/RNA
1614	Tyrosine and Tryptophan C=C stretching; Adenine	Protein; DNA
1669	Amide I	Protein

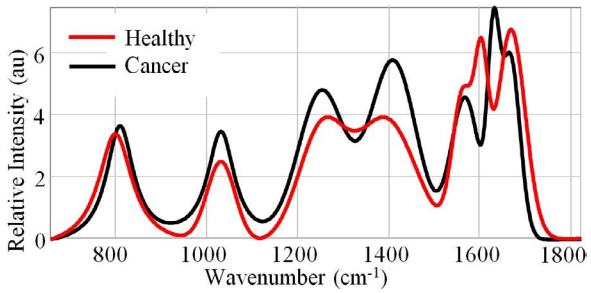
Interestingly, the Raman bands have been assigned to vibrational modes of biomolecules which have previously been shown in the literature as playing a role in identifying prostate cancer. Many different protein biomarkers have been determined for detecting prostate cancer, including the prostate-specific antigen.[64,65] Collagen metabolism has been shown to be affected due to occurrence of prostate cancer, with the observed changes related to the grade of the tumor.[66-68]

The spectra of the two cell lines also reflect a decrease in glycogen and alterations in nucleic acid content, both of which have been previously reported by Crow et al as potential Raman spectroscopic markers for prostate cancer.[69,70] Various alterations in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) have been identified for signaling presence of the cancer, including in *PCA3* and the *TMPRSS2-ERG* gene fusion.[71]

The ability to detect variations in these classes of biomolecules indicates that UVRS is sensitive to the biochemical changes that occur during the pathogenesis of prostate cancer. These identified biomolecules serve as potential spectroscopic markers for cancer and should be further studied.

Next, sections of mouse brain tissues were analyzed. Figure 2 shows the spectra for cancerous and healthy tissues.

Figure 2. *Average Spectra Collected From Water Dispersions Of Healthy Brain Tissue (Red) And Cancer Brain Tissue (Black) Of A Mouse Using Deep-UV Raman Scattering*



The spectra show great evidence of protein contribution, as designated by the Raman bands at 1260, 1400, 1602, 1630, and 1666 cm^{-1}.[57,72,73] Notably, the bands at 1630 and 1666 cm^{-1} differ greatly in intensity between the two average spectra. These bands correspond to the Amide I vibrational mode,[57] and the shift in wavelength indicates that the secondary structure of proteins in the tissue are generally undergoing a conformational change, suggesting proteins play a role in identifying brain cancer. In fact, many studies have already discovered proteins useful for identifying the presence or absence of brain cancer including thymosin β4, S100 calcium-binding protein A4, glial fibrillary acidic protein, epidermal growth factor receptor, peroxiredoxin 4, aldolase C fructose-biphosphate, and creatine kinase, to name a few.[74-76]

The influence of DNA and RNA to the Raman spectra of the two brain tissue areas are most likely denoted by peaks seen around 805 and 1570 cm^{-1}, and phospholipids contribute at 1032 cm^{-1}.[61,77-79] Nucleic acids and phospholipids have already been identified in previous Raman spectroscopic studies for contributing toward the identification of brain cancer within tissue samples.[80,81] Other studies have suggested certain RNA expression patterns as indicative of brain metastasis including ERCC1 and ERCC2 and long noncoding ribonucleic acid metastasis-associated lung adenocarcinoma transcript 1 (RNA MALAT1).[82,83] A full list of tentative Raman shift assignments for the brain tissue is presented in Table 2.

Table 2. *Tentative Raman Shift Assignments of Spectra Collected from Mouse Brain Tissue Using UVRS*

Shift (cm^{-1})	Assignment	Contribution
805	Uracil-based ring breathing; O-P-O stretching	RNA
1032	CH_2CH_3 bending	Phospholipids
1260	Amide III	Protein
1400	Aspartic and Glutamic acid (C=O) O^-stretching	Protein
1570	Guanine and Adenine ring breathing	DNA/RNA
1602	Phenylalanine and Tyrosine C=C in-plane bending	Protein
1630	Amide I	Protein
1666	Amide I	Protein

DISCUSSION

Raman spectroscopy, in general, has been widely studied for cancer identification purposes.[4,84,85] While a plethora of studies show the usefulness of near-IR and visible excitation of biological samples for detecting cancer, the advantages of UVRS spectroscopy have yet to be explored in terms of sensitivity, specificity and diagnostic accuracy. The UVRS approach is a promising and superior diagnostic method for cancer recognition due to the following factors. The excitation of biological samples in the wavelength range around 210-300 nm allows for the resonance enhancement of scattered light due to the amide chromophore, which is a crucial component of the backbone of a polypeptide; specifically, the polypeptide side chain including aromatic amino acids contributes to this enhancement.[86] The absorption spectra of aromatic amino acids typically exhibit peaks around 210-280 nm.[87] This overlap between absorption and excitation wavelengths result in the resonance enhancement of the Raman signal.[88,89] Aromatic amino acids have an inherent ability to probe the structure of the protein; with enhancement of these signals, the secondary structure of proteins is much more easily elucidated, allowing for this novel and specific information to aid in identifying malignancies. The resonance enhancement of nucleic acids has also been observed using deep-UV excitation. Mononucleotides exhibit resonance Raman enhancement at 266, 240, 218, and 200 nm UV radiation,[90,91] with various vibrational modes of the purine and pyrimidine bases observed in the resulting Raman spectrum. Again, the enhancement occurs due to the overlap between absorption and excitation wavelengths.[92] This information, which is not as well observed using near-IR or visible light excitation, provides a more specific insight into the biochemical changes that are occurring during the pathogenesis of cancers. Capitalizing on these advantages allows for a much greater potential to identify cancer within biological samples.

This proof-of-concept study contemplates the differences between single donors for two different types of cancer. While inherent inter-patient variability does exist, previous studies which have included multiple donors in each group have shown that the inter-patient variability is not significant enough to affect disease diagnostic efforts.[5,6] Furthermore, the Raman spectra for the four different biological samples shown here are dominated by protein and nucleic acid contributions. The inherent enhancement by deep-UV excitation provides crucial information regarding the differences which exist between healthy and cancerous biological samples. These differences can be translated to crucial biochemical information regarding cancer pathogenesis and may be useful for identifying cancer within patients in future studies.

Safety Considerations

There are great advantages for using UV excitation lasers, including eliminating broadband tissue autofluorescence, which arises after 255 nm (Figure 3) due to aromatic amino acids,[94,95,48] and increasing RS signals from proteins[97] and from lipids and fatty acids.[97,98]

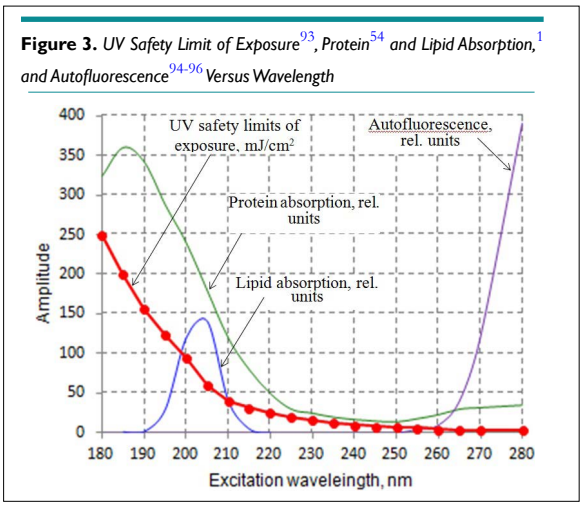


Figure 3. *UV Safety Limit of Exposure[93], Protein[54] and Lipid Absorption,[1] and Autofluorescence[94-96] Versus Wavelength*

However, shifting laser excitation to the deep-UV range brings about health risk issues, including photochemical damage of DNA.[99] The guidelines on safe UV exposure of human tissue/skin established by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) indicate that exposure doses must be limited to 10 mJ/cm^2 for 240 nm and can be as high as 250 mJ/cm^2 for 180 nm (see red line, Figure 3).[93,100,101]

In this study, the UV radiation of a 0.4 mW laser was delivered and focused into a 7-μm spot (area ~50 μ^2) in the middle of a quartz tube filled with a water dispersion of either tissue or cells; the relative volume concentration of the cells was 0.5% (1:200). The UV Raman signal in our experiment was collected for 30 seconds using an optical system with 8% collection efficiency, and recorded by a UV CCD camera with an estimated detection efficiency of 5%.[60] Taking into account the small (0.5%) volume concentration of the cells in our experiment, we can derive, in the case where cells cover the entire sample area of 50 μ^2, the same level of Raman signal will be achieved after 30 s/200=0.15 s collection time. Accordingly, the energy density (i.e., energy per cm^2) of UV radiation for this area is 4×10^{-4} W$\times1.5\times10^{-1}$ s/(50×10^{-8} cm^2)=120 J/cm^2.

In future studies, the goal is to translate this work into the development of an efficient fiber-optic probe for UVRS-based cancer diagnostics of living tissues, which could map a living tissue in real time. As such, it is necessary to ensure that excitation by a deep-UV laser will not cause physiological problems. Assuming

that the sample area illuminated by the Raman probe is 300×300 μm^2 (~2000-fold larger than illuminated area in this study) and that photon collection efficiency of the Raman probe is approximately 20% we can calculate that, using the same UV laser, the radiation dose in the proposed approach is only 1:5000 of that in this study. This is equivalent to 120 $J/cm^2/5000=25$ mJ/cm^2, which is considered safe according to the ICNIRP guidelines discussed above.

To the best of our knowledge, this feasibility study demonstrates for the first time the ability of deep-UV Raman spectroscopy to discriminate cancerous from normal tissues and cells in a relatively short time with high sensitivity. The dramatic increase in sensitivity observed in our experiments, which cannot be obtained using near-IR or visible excitation, allows for identification of altered chemical/structure composition of cancerous versus normal conditions. This result may lead to a better understanding of changes which occur in a biochemical environment during the pathogenesis of cancer. The clear advantages of this method suggest that UVRS should be considered in future studies as a novel methodology for the specific and informative identification of cancer. Because it also meets safety guidelines for UV exposure, this method has the potential to be further developed into a real-time diagnostic method using fiber-optics.

CONCLUSION

This proof-of-concept study suggests deep-ultraviolet resonance Raman spectroscopy has significant potential for identifying cancerous and healthy biological species at both the cellular and tissue level. Many key advantages exist for using deep-UV excitation as compared to visible or near-IR excitation. Notably, deep-UV excitation provides resonance enhancement of crucial biochemical components and eliminates interference from fluorescence. These advantages allow for simple spectral interpretation, saving time and effort for the researcher which can be critical in diagnostic situations. Notably, changes in biochemical composition between healthy and prostate and brain cancer samples were discovered using UVRS. These differences can be capitalized on in future studies with larger sample sizes in order to further develop the method for identifying prostate and brain cancers. The proposed approach is shown to be a safe and effective method for detecting cancer within biological samples and provides a solid basis for research into further developing UVRS for clinical applications.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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Review

Anticancer Natural Products: A Review

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ABSTRACT

Historically, natural products played a forceful role in human treatment ailments. Nowadays, natural products include a large part of current pharmaceutical agents, mostly in the field of cancer therapy. The main aim of this review is to provide a comprehensive summary of the most known natural product used as anticancer globally, including various other natural products. Many of these natural product appears to act through an anticancer mechanism. Overall, natural product research is a vigorous tool to discover novel biologically active components with unique mechanisms of action. Given the diversity of nature, it is sensible to indicate that chemical leads can be produced that are able to interact with most therapeutic targets. This review creates a solid foundation for further study these natural products with additional research and study.

Keywords
Anticancer; Natural product; Plant compounds; Marine flora; Microorganisms; Venom.

INTRODUCTION

Cancer is a serous global health problem responsible for millions of deaths all over the world. It is responsible for approximately 7.6 million deaths worldwide, which is expected to increase to 13.1 million by 2030.[1] Despite the progress in the field of cancer research, still there is a need to discover and develop anti-cancer therapeutic agents. Since long it has been recognized that, natural products represent the richest source of high chemical diversity, providing the basis for identification of novel scaffold structures that serves as starting points for rational drug design.[1] This can be one of the reasons that efforts have been directed to discover promising cancer therapeutic agents from natural sources. Over the years, many natural product-based drugs have been introduced in the market.[2] According to a recent review, 49% of drugs were either natural products or their derivatives that are used in cancer treatment.[3] Moreover, between the year 2005 and 2010, nineteen natural product-based drugs have been approved, among which seven have been classified as natural product, ten as semi-synthetic natural product and two as natural product-derived drugs.[4] Of these, five drugs, everolimus, temsirolimus, ixabepilone, trabectedin and romidepsin, have been developed in the feild of oncology from 2007 to 2009.[1]

Natural products comprise any substance produced by life organism. Mostly, these substances are of small molecular weight (<3,000 Daltons) and of considerable structural diversity. Over 40-years, natural products played a powerful role as established cancer chemotherapeutic agents, either in their naturally occurring forms or their synthetically modified forms.[5] For example, antitumor antibiotics from microbes include the anthracyclines (such as doxorubicin), bleomycin, dactinomycin (actinomycin), and mitomycin C. In turn, members of four classes of plant-derived compounds are used widely as antitumor agents, namely, the bisindole (vinca) alkaloids, the camptothecins, the epipodophyllotoxins, and the taxanes.[6] In addition, there are several examples of promising natural product-derived antineoplastic agents currently in advanced clinical development or recently approved, not only from microbes (e.g., the epothilones and the enediynes) and plants (e.g., the combretastatin and homoharringtonine analogs), but also of marine origin (e.g., the bryostatins, ecteinascidin 743, kahalalide F).[5] Of a total of 155 anticancer agents approved for use in Western medicine and Japan since the 1940s, 47% were classified as either natural products (14%) semi-synthetic derivatives of natural products (28%), or otherwise derived from natural products (5%).[5] Among the largest groups of taxonomically identified classes of organisms that may be studied as sources of new anticancer drugs are arthropods, higher plants, and marine

invertebrates.[7] In addition, natural product researchers have examined other taxonomic classes of organisms found all over the world, including algae, bacteria, fungi, and even terrestrial vertebrates.[5] Natural product drug discovery for anticancer agents requires special procedures involved with sample collection, inclusive of the development of "benefit-sharing" agreements with source countries, whether the samples are of marine or terrestrial origin.[8]

There is a tendency for natural product chemists to specialize on the types of organisms they work, such higher plants or marine fauna, due to the different methods of organism collection and work-up in the laboratory.[5] However, there is increasing evidence that the same secondary metabolite of significance as a potential anticancer agent may be produced by more than one type of organism.[9]

Plant Compounds with Anticancer Properties

The plant based drug discovery give rise to the development of anticancer agents, including plants (paclitaxel, etoposide, camptothecin, vinblastine, vincristine, topotecan, and irinotecan). Beside this there is various agents identified from fruits and vegetables can used in anticancer therapy (Table 1) include spices yielding biologically active components such as curcumin, lycopene, saponins, isoflavones, cucurbitacins, phytosterols, resveratrol, and others.[10] There are compounds which have been identified and extracted from terrestrial plants for their anticancer properties include alvaradoin E (bioactivity-directed fractionation of an extract of the leaves of alvaradoa haitiensis Urb. (picramniaceae).[11] Pancratistatin 3,4-O-cyclic phosphate sodium salt (pancratistatin, a phenanthridone alkaloid, from the bulbs of the plant *Pancratium littorale Jacq*. (Amaryllidaceae)).[12] Polyphenolic compounds include (flavonoids which constitute a large family of plant secondary metabolites as anthocyanins, flavones, flavonols and chalcones[13]; tannins[14]; curcumin[15]; Resveratrol which found in foods including peanuts and grapes and red wine[16] and gallacatechins which present in green tea.[17] Brassinosteroids are naturally occurring compounds found in plants which have role in hormone signalling to regulate growth and cell differentiation, stem and root cells elongation and other roles such as tolerance against disease and stress.[17]

Table 1. *List of Important Anticancer Plant Compounds and Its Mechanism of Action*[18]

S. No.	Scientific Name	Administration of Drug (Compound/Crude Extract) to Experimental Model	Mechanism of Action
1	*Acacia catechu (L.f.) Willd.*	100 µg /ml of catechin rich extract (AQCE) was used against MCF-7 (Human breast adenocarcinoma cellline)	Down regulation of NF-κB and AP-1 expression (cell differentiation and proliferation). Decreases c-jun expression
		10-100 µg /mL of 70% methanolic extract (ACME) from heartwood acts against 7, 12-di methyl benz[a] anthracene induced mammary carcinoma in Balb/c mice.	Induces cell cycle arrest at subG1 phase by increasing Bax/Bcl2 ratio and activating caspase cascade which leads to the cleavage of poly adeno ribose polymerase (PARP)-intrinsic pathway
2	*Allamanda cathartica L.*	Allamandin, β-amyrin, plumericin, isoplumericin, β sitosterol and ursolic acid from leaves through molecular docking	Inhibit cyclin dependent kinases (CDK1) protein regulates cell cycle
3	*Aloe barbadensis Miller.*	200 µmol/L of aloin from leaves was used against HUVECs (human umbilical vein endothelial cells) and SW620 (human colorectal cancer cells)with the dosage of 20 µmol/L	Apotosis and anti-angiogenesis: Suppresses activation of VEGF receptor (VEGFR) 2 mediated c-src and JAK2. Phosphorylation of STAT3 in endothelial cells. Down-regulates activated STAT3 protein, expression of STAT3-regulated antiapoptotic (Bcl-xL), proliferative (c-Myc) proteins.
4	*Anisomeles indica L.*	40 µM of ovatodiolide against renal cell carcinoma	Inhibits β-catenin signaling
		500 µg/mL of aqueous extract from whole plants and 30 µM apigenin was used against 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced MCF-7 cells (Human breast adenocarcinoma)	Anti-metastasis, anti- migration and anti- invasion: Downregulates matrix metalloproteinase (MMP)-9 enzymatic activities, mRNA expression, nuclear factor (NF)-κB subunit p65 and activator protein (AP)-1 subunit c-Fos proteins expression in nucleus
		10, 20 and 40 µM of ovatodiolide from whole plant were used against MDA-MB-231[73]	Cancer cell growth inhibition and proliferation: Prevents phosphorylation of upstream signal IκB kinase. It also suppresses activation of c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, phosphatidylinositol 3-kinase and Akt
5	*Bauhinia racemosa L.*	Methanol extract from stem bark used against N-nitrosodiethyl amine (NDEA) induced hepato carcinogenesis in wister albino rats	Chemoprevention: It suppresses nodule development or hepato cellular lesion formation. It decreases lipid peroxidation and enhances antioxidants levels by reducing the formation of free radicals.
		50, 100 and 200 mg/kg of methanolic extract from stem bark against ehrlich ascites carcinoma (EAC) in swiss albinomice	Before treating drug: Increased level of serum enzymes, bilirubin and decreased protein and uric acid level. Elevated amount of MDA (malondialdehyde) decreased level of antioxidants.
6	*Bauhinia variegata L.*	Ethanol extract from bark and stem were used against HeLa, Dalton's ascetic lymphoma, leukemia and ovariancancer	Arrest G0/G1 phase
7	*Butea monosperma L.*	100 mg/kg and 25 mg/kg of aqueous extract from flower acts against Huh7 and HepG2 cells (hepatoma cells)	Arrest in G1 phase down-regulates MAP kinase and SAPK/JNK signaling pathways
8	*Cajanus cajan L.*	15 or 30 mg/kg of cajanin stilbene acid was used against MCF-7	Induce G2M arrest and apoptosis by activating the mitochondrialpathway
		64 µM of cajanol (5-hydroxy-3-(4-hydroxy-2- methoxyphenyl)-7-methoxychroman-4-one) from root	ROS-mediated mitochondria-dependent pathway induces G_2/M phase and apoptosis inhibits expression of Bcl-2 and induction bax expression leads to activation of caspase-9 and caspase-3 cascade, which is involved in PARPcleavage

9	*Calotropis gigantea L.*	1, 5 and 10 nM of cardenolides and calotropin from root bark used against DLD1, HCT116 and SW480 99	Phosphorylation and degradation of β-catenin by casein kinase 1α inhibits Wnt signaling.
10	*Cardiospermum halicacabum L.*	< 20 µg/ml of n-hexane extract from seeds was used against MCF-7 (Breast cancer cell line)	Anti-proliferative activity
11	*Cissus quadrangularis Linn.*	Acetone extract from stem used against A431 (Human skin epidermoid carcinoma) cellline[21]	Bax–Bcl2 ratio, release of cytochrome c from mitochondria to cytoplasm, cleavage of PARP
12	*Curcuma zedoaria C.*	500 mg/kg of isocurcumenol was used for A549 (Lung carcinoma), KB (nasopharyngeal carcinoma), K562 (leukemic), daltons lymphoma ascitescells	Immuno modulation, immuno stimulation, effects on humoral immune response, anti-angioneogenesis activity
		400 µM of α-curcumene from Rhizome acts against SiHa cells (Human ovarian cancer)	Mitochondrial cytochrome c complex with Apaf-1 and pro-form of caspase-9 activates caspase-3 and caspase-9.
13	*Dioscorea bulbifera L.*	30 mg/ml of ethyl acetate soluble fraction of 75% ethanol extract of the rhizomes was acts against JB6 (Mouse epidermal) cell lines induced by 12-O-tetra de canoylphorbol-13-acetate (TPA)	Onco-protein kinase activation and reactive oxygen burst
14	*Drosera indica L.*	250, 500 mg/kg of ethanol and 500 mg/kg of aqueous extract from whole plant used against dalton lymphoma ascites (DLA) cells in male and female adult swiss albino mice	Increases caspase-3 activity and decreases DNA, RNA and protein content. Cell growth inhibition through antioxidant property
		250 mcg/ml ethanol and aqueous extract was used against ehrlich ascitic carcinoma (EAC) cell line	Anti tumour: Lactate dehydrogenase (LDH) leakage and increased scavenging effect
15	*Elephantopus scaber L.*	25, 50, 100 and 200 µg/ml dichloromethane fraction from whole plant was act against HeLa (cervical), A549 (lung), MCF7 (breast) and Caco2 (colon)	Apoptosis: Enhanced sub G0 content and micronuclei formation. Genotoxicity. Inhibited MDR transporters (ABCB1 and ABC G2)
16	*Embelia ribes Burm.*	10-30 µM of embelin from fruits used against MCF7	Reduction in TNF-α and synthesized as pro- TNF-α then released to extra cellular space by TNF-α converting enzyme.
		Embelin from fruits used for molecular docking (breast cancer cells)	Inactivation of metastatic signaling: MMPs,VEGF and hnRNP-K transcriptional attenuation of mortalin and activation of p53
17	*Gymnema sylvestre R.Br*	121µg and 250 µg of aqueous extract from leaves was used against Hep2 (Liver cancer) cells	Anti-proliferation: Increases intracellular ROS levels
18	*Jatropha gossypifolia L.*	10 µg/ml of whole plant ethanolic extract acts against MCF-7 (Breast cancer cells)	Pro-apoptotic and anti-adhesive effects: Decreases β1- integrin expression and phosphorylation of the focal adhesion kinase at Tyr397
19	*Kaempferia galanga L.*	Ethyl p methoxy cinnamate from Rhizome was used against HepG2 cells (Human hepatocellular liver carcinoma)	Apoptotic induction and inhibition of proliferation: Increase subG0 cell population
20	*Kaempferia rotunda L.*	500 mg/Kg of chloroform extract and 20 mg/Kg of pinostrobin from Rhizome acts against T47D (Human breast cancer cell lines)	Suppress c-Myc expression
21	*Lantana camara L.*	15 mM of pentacyclic triter penoids-reduced Lantadenes A and B used against HL-60 cells.	Induction of apoptosis: Suppresses the production of nitrite, TNF-α and iNOS gene expression
		20, 40, 80 mg/kg of Ursolic acid stearoyl glucoside act against Induced hepato cellular carcinoma in wistar rats by diethylnitrosamine (DENA).	It suppresses free radical formation by scavenging the hydroxyl radicals. Modulates the level of lipid peroxidation and increases the endogenous antioxidant enzymes level
		30 µg/mL of ethanolic extract from Leaves act against MCF-7 (Human breast cancer cell line)	Bid and bax was increased and Bcl-2 was decreased after drug treatment. It also modulates cleavage of caspase-8, caspase-9 and poly (ADP-ribose) polymerase(PARP)
22	*Lawsonia inermis L.*	30 µg/ml-1 of leaves chloroform extract act against Hep2 cells and Caco2 (colon)	Down regulation of c-myc expression
		180 mg/kg of ethanolic crude extract from root was used against Dalton's lymphoma ascites.	Enhances the activities of catalase, glutathione peroxidase and glutathione S transferase and increases vitamin C, E and reduced glutathione level.
23	*Leea indica Burm.*	40 mg/kg/day of methanolic extract acts against ehrlich ascites carcinoma (EAC) cells in swiss albino mice	cytotoxicity
		60 µM of mollic acid arabinoside was used against Ca Ski cervical cancer cells	Induce mitochondrial mediated apoptosis
		60 µM of mollic acid xyloside (MAX) from leaves against Ca Ski cervical cancer cells	Decreases the expression of proliferative cell nuclear antigen, increases sub-G1 cells and arrest cells in S and G2/M phases
		500 and 1000 µg/mL of ethyl acetate fraction was used against Ca Ski cellline	Inducing apoptosis: Accumulation of sub-G1 cells, depletion of intracellular glutathione and activation of caspase-3.
24	*Moringa oleifera L. Moringaceae*	50 µg/ml of ethanolic extract from leaves, bark and seed showed activity against MDA-MB-231 and HCT-8 (colorectal)	Anti-malignant properties: Arrest cell
		50-400 µg/ml of leaf extract against HepG2 (Hepato cellular carcinoma cells) and A549 non-small cell lung cancer	Anti-proliferation and apoptosis
25	*Oroxylum Indicum L.*	20 µM of baicalein from stem bark against CT-26 (colon carcinoma)	Inhibit activation of pro-PDGF-A, B and pro-VEGF C
26	*Oxalis corniculata Linn.*	100 and 400 mg/kg of Ethanolic extract from Whole plant for Ehrlich ascites carcinoma (EAC)-induced in swiss albino mice	Antitumor and antioxidant activity: Increase intotalprotein, albumincontent,catalaseand reduced glutathione levels. Decrease in AST, ALT and ALP contents, liver MDA level

27	*Physalis minima L.*	25-100 g/ml of chloroform extract from whole plant was used against NCI-H23 (Human lung adenocarcinoma)	Inhibit cell proliferation and induce apoptosis: Activation of c-myc, caspase-3 and p53 gene expression
		6.25 µg/mL of Physalin F used against T-47D cells (Human breast carcinoma)	Chemoprevention / apoptosis: Activation of caspase-3 and c-myc pathways due to the presence of cyclo hexanone and epoxy moieties.
		25-100 g/ml of chloroform extract from whole plant act against Caov-3 (Human ovarian carcinoma)	Apoptosis and autophagy
28	*Polyalthia longifolia Sonn.*	50 µg/ml of was chloroform extract from Leaves against HL-60	Induce intrinsic or mitochondrial-dependent apoptotic pathway
29	*Tecomella undulate D.*	30 µg/ml of undulatoside-A, undulatoside-B and tecomin from bark acts against K562 (chronic myeloid leukemia cells)	Cell cycle arrest at S phase, increase in Annexin V positive cells. Increase in FAS, FADD levels and activation of caspase 8 and 3/7
30	*Terminalia chebula R.*	100 µl of chebulagic acid from fruits showed apoptosis in COLO-205 cells	Inhibition activity of COX and 5-LOX
31	*Tinospora cordifolia T.*	100 and 200 mg/kg of palmatine (alkaloid) from stem against 7,12-dimethylbenz(a) anthracene (DMBA) induced skin carcino genesis in swiss albino mice.	Antioxidant and chemo-preventive activity
		100 µl of Hexane fraction act against EAT (Ehrlich ascites tumor).	Apoptosis signals activates caspase-8, its substrate BID protein releases cytochrome C to bind Apaf-1 which induces auto-activation of caspase-9, which in turn activates caspase-3. It cleaves poly-ADP-ribose polymerase, lamins and inhibitor of caspase activated DNase (ICAD).
32	*Triumfetta rhomboidea Jacq. (Tiliaceae)*	100 and 200 mg/ kg of leaves methanolic extract used against ehrlich ascites carcinoma (EAC) DLA bearing male swiss albino mice.	Antitumor and antioxidant activity: Decreases the level of lipid peroxidation and increases glutathione (GSH), superoxide dismutase (SOD) and catalase level
33	*Urginea indica Roxb.*	75 µgml-1 of Glycoprotein from bulbs act against HUVECs and EAT cells in swiss albino mice	Antiangiogenic and proapoptotic activity: Inhibition of translocation of nuclear factor kappa B to the nucleus thus decreases the expression of vascular endothelial growth factor gene
34	*Vitex negundo L. (Verbenaceae)*	10 µg/mL of chrysoplenetin and chrysosplenol D used against PANC-1 (Human pancreatic cancer) cells, NCI-H522 (lung), OVCAR-3 (ovarian) and PC-3 (prostate) cells.	Cytotoxicity and apoptotic morphological changes (DNA fragmentation, nuclear condensation and membrane blebbing)
		4 to 6 µg/mL of vitexin from i) Fruit iii) Seeds used against COC1 (ovarian cancer cells) and MDA-MB-231154	Apoptosis by caspase activates poly (ADP ribose) polymerase (PARP) and cleaved into a COOH-terminal fragment

Table 2. *Anticancer Compounds from Marine Environment*[22]

No	Name of the Compound	Source of Organisms	Chemical Class	Cancer Target
1	Arenamides A–C	Actinomycete (*Salinispora arenicola*)	Cyclohexa-depsipeptides	Human colon carcinoma cell line (HCT-116)
2	Heteronemin	Sponge (*Hyrtios sp.*)	Sesterterpene	Leukemia (K562 cells)
3	6-bromoisatin	Whelk (*Dicathais orbita*)	Indole derivative	Ovary, granulosa, Choriocarcinoma (OVCAR-3, KGN, Jar)
4	Tyrindoleninone	Whelk (*Dicathais orbita*)	Indole derivative	Ovary, granulosa, Choriocarcinoma (OVCAR-3, KGN, Jar)
5	Cryptosphaerolide	Ascomycete fungal strain CNL-523 (*Cryptosphaeria sp.*)	Sesquiterpenoid	Human colon carcinoma cell line (HCT-116)
6	Makaluvamine A	sponge (*Zyzzya fuliginosa*)	Pyrroloquinoline	Colon cancer (HCT-116 cells)
7	Ascididemin	Actinomycete (*Salinispora arenicola*)	Cyclohexa-depsipeptides	Human colon carcinoma cell line (HCT-116)
8	Lamellarin D	Prosobranch mollusc of the genus (*Lamellaria*)	Alkaloid	Leukemia
9	Spongistatin 1	Sponges (*Spirastrella spinispirulifera and Hyrtios erecta*)	Macrocyclic lactone	Leukemia (Jurkat cells)
10	Streptochlorin	*Streptomyces sp.*	Methyl pyridine	Leukemia (U937 cells)

Anticancer Compound from Marine Flora

Marine floras include microflora (bacteria, actinobacteria, cyanobacteria and fungi, microalgae, macroalgae, and flowering plants (mangroves and other halophytes) contain a massive number of natural products and novel chemical structures with unique activities that may be useful in finding the potential drugs with major efficacy and specificity forhuman treatment[19] (Table 2). The marine organisms produce novel chemicals to withstand extreme variations in their environment, and the chemicals produced are unique in diversity, structural, and functional features.[20] Mostly invertebrates that include sponges, soft corals, sea fans, sea hares, nudibranchs, bryozoans, and tunicates are proven to be the potent sources of drugs.[21] It is now believed that microbial flora present in the invertebrates are responsible for the production of medicinal compounds. Marine floras are rich in biologically active and medicinally potent chemicals as polyphenols, polysaccharides and alkaloids are the most predominant group of compounds which are applicable for antioxidant and anticancer activities.[19]

Table 3. *Some Examples of Bacterial Strains with Bioactivity and the Sources where they were Obtained*[26]

Bacteria	Gram (+ or -)	Activity	Target organism	Disease
Pseudomonas bromoutilis	-	Anticancer	*Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes*	Pneumonia, osteitis, arthritis, endocarditis, localized abscesses
Chromobacteria marinum	-	Antibacterial	*Escherichia coli, Pseudomona aureginosa, Staphylococcus aureus*	Pneumonia, osteitis, arthritis, endocarditis, localized abscesses
Flavobacteria uliginosum	-	Anticancer	Sarcoma-180 cells	Viral tumor
Bacillus sp.	+	Anticancer	HCT-116 cells	Colorectal Cancer
Lactococcus lactis	+	Anticancer	Human papilloma virus type 16(HPV-16)	Colorectal Cancer
Staphylococcus aureoverticillatus	+	Anticancer	Tumor cells	Tumors
Marinobacter drocarbonoclasticus	-	Antibacterial (siderofore)	*Mycobacteria tuberculosis, Bacillus anthracis*	Tuberculosis, carbuncle (anthraxlike)

Marine bacteria: Produce secondary metabolites which have anticancer agents (e.g., eleutherobin, discodermolide, bryostatins, and sarcodictyin)[23] as in Table 3. Most of marine bacteria produces toxins which are useful in neurophysiological and neuropharmacological studies.[24] Only a few marine bacteria can be isolated under laboratory conditions and there is an urgent need to isolate the bacteria that produce unique and novel natural products.[25]

Marine actinomycetes: Received very recent attention. Gutingimycin is a highly polar trioxacarcin derivative from streptomyces species, isolated from sediment of the Laguna de Terminos, Gulf of Mexico.[19] The same Streptomyces species also yields trioxacarcins D-F, in addition to the known trioxacarcins A-C. Among the antibiotic-producing microbes, marine actinomycetes within the family micromonosporaceae are very promising.[27] These microbes revealed to be a promising sources of anticanceragents that target proteasome function.

Thiocoraline is a novel bioactive depsipeptide isolated from *Micromonospora marine*, a microorganism located in the mozambique strait that inhibits ribonucleic acid (RNA) synthesis.[28]

Marine fungi: Marine fungi are least studied than terrestrial fungi. Obligate marine fungi are still an unexplored resource, although, marine facultative fungi, have been studied due to their production of new metabolites which are not found in terrestrial fungi.[29] Recently more interest has been generated on studying biologically active metabolites from higher fungi (basidiomycetes), endophytic fungi and filamentous fungi from marine habitats, the symbiotic lichens on its anticancer activity.[30]

Marinemacro algae (Cyanobacteria): Marinemicro algae is one of the potential organisms which can be the richest sources of potent bioactive compounds including toxins with potential for pharmaceutical applications.[31] More than 50% of the marine cyanobacteria are potentially exploitable for extracting bioactive substances which are effective in killing the cancer cells.[19] Scytonemin is a protein serine/threonine kinase inhibitor isolated from the cyanobacterium *Stigonema sp.* and this compound is a yellow-green ultraviolet sunscreen pigment, known to be present in the extracellular sheaths of different genera of aquatic and terrestrial blue-green algae.[23] Largazole derived from *Symploca sp.* is a novel chemical scaffold with fabulous antiproliferative activity.[19] Other compounds, apratoxin A, isolated from a strain of Lyngbya boulloni,[32] coibamide A derived from a strain of Leptolyngbya,[33] curacin-A, isolated from the organic extracts of curacao collections of Lyngbya majuscule.[34]

Marine macro algae (Seaweed): Marinemacro algae many researchers have worked on the antioxidant, antitumor, and immunomodulating activities of seaweeds as edible seaweed like *Palmaria palmate*,[35] the alcoholic extract of the red alga *Acanthophora spicifera*,[36] the seaweeds *Acanthaphora spicifera*,[37] *Ulva reticulate*,[38] *Gracilaria foliifera*,[39] the brown seaweed *Sargassum thunbergii*,[40] fucoidan from *Ascophyllum nodosum*,[41] stylopoldione from *Stypodium sp.*,[19] condriamide-A from *Chondria sp.*,[42] caulerpenyne from *caulerpa sp.*,[43] two compounds meroterpenes and usneoidone isolated from *Cystophora sp.*,[44] phloroglucinol and its polymers namely eckol (a trimer),[45] *phlorofucofuroeckol* A (a pentamer),[45] dieckol and 8,8'-bieckol (hexamers) isolated from the brown alga eisenia bicyclis and padina[45] owing to their biological properties.

Mangroves and other higher marine plants: Mangroves have long been used in fisher-folk medicine to treat diseases. Based on traditional knowledge and preliminary scientific work, sixteen higher marine plants considered asa source of anticancer drugs[19] (Table 4). A sulphur containing alkaloid, 1,2-dithiolane (brugine) isolated form *Bruguiera sexangula*, ribose derivative of 2-Benzoxazoline isolated from *Acanthus ilicifolius* and tea from the mangrove plant *Ceriops decandra* has shown anticancer activity.[46]

Microorganisms with Anticancer Properties

Small organic molecules derived naturally from microorganisms have provided a number of beneficial cancer chemotherapeutic drugs.[5] Introduce microorganisms into the body leads to the activation of various immune mechanisms, which manifests itself in increasing the number and recruitment of congenital immune cells, activation of acquired immunity cells, and production of proinflammatory cytokine.[48] It is assumed that the rallied immune system, by intentionally introducing microorganisms into the oncological patient, is able to at least limit the development

Table 4. *List of Anticancer Compounds Isolated from Endophytic Fungi from Mangrove Habitats*[47]

No	Host Plant	Fungal Endophyte	Isolated Cytotoxic Compound/s	Tested Cell Line/s	Cytotoxicity
1	*Excoecaria agallocha*	*Phomopsis sp.* ZSU-H76	2-(7'-hydroxyoxooctyl)-3-hydroxy-5-methoxybenzeneacetic acid ethyl ester	HEp2	25
				HepG2	30
2	*Rhizophora mucronata*	*Pestalotiopsis sp.*	Cytosporones J-N	L5178Y	Not Active up to 10 μg/mL
			Pestalasins A-E	HeLa	
			Pestalotiopsoid A	PC12	
3	*Rhizophora mucronata*	*Pestalotiopsis sp.*	Pestalotiopsone A	L5178Y	NA
			Pestalotiopsone B		NA
			Pestalotiopsone C		NA
			Pestalotiopsone D		NA
			Pestalotiopsone E		NA
			Pestalotiopsone F		26.89
4	*Not mentioned*	*Mangrove endophytic fungus No. ZSU44*	Secalonic acid D	HL60	0.38
				K562	0.43
5	*Excoecaria agallocha*	*Pestalotiopsis sp.*	Phomopsis-H76 A	KB	Allthecompoundsare
			Phomopsis-H76 B	KBv200	Inactiveagainstallthe
			Phomopsis-H76 C	MCF7	Tested cell lines
6	*Kandelia woody tissue*	*Halorosellinia sp.* *Guignardia sp.*	1-hydroxy-3-methyl anthracene-9,10-dione	KB	3.17
				KBv200	3.21
7	*Sonneratia apetala*	Zh6-B1 (unidentified)	3R,5R-Sonnerlactone	KV/MDR	42.4
			3R,5S-Sonnerlactone		41.6
8	*Xylocarpus granatum*	XG8D (unidentified)	Merulin A	BT474	4.98
				SW620	4.84
			Merulin B	BT474	>10
				SW620	>10
			Merulin C	BT474	1.57
				SW620	4.11
9	*Acanthus ilicifolius*	*Pestalotiopsis sp.*	Penicinoline	95-D	0.57
				HepG2	6.5
				HeLa	>100
				KB	>100
				KBv200	>100
				HEp2	>100
10	*Unidentified mangrove (Taiwan Strait)*	*Pestalotiopsis sp.*	Paeciloxocins A	HepG2	1
			Paeciloxocins B		65
11	*Excoecaria agallocha*	*Penicillium expansum*	Expansols A	A549	NR
				HL-60	15.7
			Expansols B	A549	1.9
				HL-60	5.4
12	*Kandelia candel*	*Fusarium sp.*	5-O-methyl-2'-methoxy-3'-methylalpinumisoflavone	HEp2	4
				HepG2	11
13	*Aegiceras corniculatum*	*Alternaria sp. ZJ9-6B*	Alterporriol K	MDA-MB-435	26.97
			Alterporriol L	MDA-MB-435	13.11
14	*Rhizophora mucronata*	*Irpex hynoides*	Ethyl acetate extract	HEp2	125
15	*Rhizophora annamalayan*	*Fusarium oxysporum*	Taxol	NT	NT
16	*Bruguiera gymnorrhiza*	*Rhytidhysteron rufulum*	Rhytidchromones A	MCF7	19.3

Compounds are included in the column"isolatedcompound/s". NA-Not Active; NR-Not Reported; NT-Nottested

of cancer.[49] This is a method in which microbes indirectly lead to cancer regression especially in those in whom other commonly used treatments have failed.

Bacteria: Bacteria can be applied in various forms for therapeutic purposes. Apart from the whole, living attenuated cells, we can use genetically engineered bacteria expressing particularly desirable factors.[50] Microorganisms are also applied as vectors, which are carriers of specific chemotherapeutics agents or enzymes useful in thedestruction of cancer cell. This method allows a significant reduction of the side effects of treatment that usually accompany traditional chemotherapy.[51] Moreover, there is a therapeutic potential to use bacterial secretion, for example, toxins.[52] Their presence in the tumor environment could have destruct the cancer cells. The use of sporangial bacteria, which can survive under unfavorable environmental conditions, represents another approach, which has been applied in the experiments with *Clostridium novyi*. This microorganism prefers anaerobic conditions, which are found in the tumor.[53] Instead of spreading over the entire organism, the bacteria are directed to the tumor site only, where they have the optimal conditions for growth.[54] This bacterial property allows the patient to be protected against the development of serious infections. From the bacteria that used in cancer therapy (*Mycobacterium bovis* BCG is a strain of mycobacterium bovis developed by Albert Calmett and Camille Guérin as a tuberculosis vaccine[55]; *Streptococcus pyogenes OK-432*[56]; *Clostridium novyi*[57]; *Salmonella enterica*[50]; serovar typhimurium which is obligate anaerobes and facultative anaerobes[58]; *Clostridium histolyticum*[59]; *Magnetococcus marinus* MC1 is a gram-negative cocci found in the Atlantic Ocean near Rhode Island, USA.[60]

Toxoplasma gondii: Toxoplasma gondii is an obligatory intracellularparasite.[61] It is life-threatening to people with impaired immunity or pregnant women, who can suffer abortion or birth malformation. It turns out that the protozoan and its lysate, toxoplasma lysate antigen, can be used to treat cancer.[60]

Plasmodium falciparum: Plasmodium falciparum (Malaria) caused by *Plasmodium sp.*, is one of the most common parasitic diseases in the world.[62] *Plasmodium falciparum* is considered to be the most malignant causative agent of malaria because it aggregates erythrocytes and thrombocytes that adhere to the vascular endothelium, which can lead to the closure of vascular light and thus damage to vascular walls and even necrosis. However, despite all the negative features of the parasite, it can be used to treat cancer.[63]

Natural Product with Anticancer Activity from Terrestrial Vertebrate and Invertebrate

Mammals and milk: Natural product isolated from mammal source is poorly studied, throughout screening for the review little data were available. Ryan et al[64] described four bovine meat-derived peptides that inhibit angiotensin-converting enzyme (ACE) and also exhibit anti-proliferative activity. A number of studies have reported the anticancer effects of milk protein-derived peptides on various cancer cells as the casein fraction-derived caseinophosphopeptides (CPPs) and lactoferrin is an 80-kDa iron-binding glycoprotein that belongs to the transferrin family.[65]

Amphibians: Amphibians skin secretions contain a wide range of biologically active compounds and have garnered attention due to their potential for drug development.[66] Moreover, the Chinese traditionally administered secretions from frog skin and toad parotid glands for medicinal purposes since ancient times. Hundreds of those peptides have been identified since the discovery of the first antimicrobial peptide from amphibian skin. Some of the naturally occurring amphibian skin peptides and their analogs proven to be cytotoxic to tumor cells only and are promising anticancer agents for example, Alyteserin-2a, isolated from the midwife toad (*Alytes obstetricans*)[67]; ascaphin-8 and XT-7 peptides obtained from the skin secretions of *Ascaphus truei* and *Silurana tropicalis*[68]; aurein peptides from the green and golden bell frog (*Litoria aureus*) and the southern bell frog (*Litoria raniformis*)[69]; dermaseptin B2 and B3,of the dermaseptin family, isolated from the South American tree frog (*Phyllomedusa bicolor*)[70]; dermaseptin L1 and phylloseptin L1, isolated from the lemur leaf frog (*Agalychnis lemur*).[71]

Reptilian: Reptilian peptides derived from crocodiles as the cationic antimicrobial peptides KT2, RT2 and RP9 from *Crocodylus siamensis* leukocyte extract proven to have a great anticancer activity.[72] He et al[73] has reported antitumor peptides T1 and T2 derived from the enzymatic hydrolysates of the Chinese three-striped box turtle (*Cuora trifasciata*).

Animal venoms: Animal venoms and toxins consist of a complex mixture of proteins and peptides and are rich with biologically active peptides with potent anticancer activity.[74] Among venomous animals, scorpions, is a source of peptidyl neurotoxins, which are used as tools to study different ion channels, such as the Na^+, K^+, Ca^+, and Cl^- ion channels[75] (Table 5). Chlorotoxin (CTX) is a small neurotoxin of 36 amino acids that was isolated from the venom *Leiurus quinquestriatus* scorpion. Initially, CTX was used as a pharmacological tool to characterize chloride channels. CTX can target glioma, small cell lung carcinoma, melanoma, neuroblastoma and medulloblastoma cells.[76]

Spider venom contain proteins and peptides including enzymes (such as proteases, phospholipases, andhyaluronidases), neurotoxins, and cytolytic peptides.[77] A short cationic peptide latarcin 2a (Ltc2a) isolated from *Lachesana tarabaevivenom*[78] have anticancer activity.

Venom from bees and wasps is now being studied to design and develop new therapeutic drugs from their venom.[79] Melittin peptide (26 amino acid) isolated from the honey bee *Apis mellifera*, is the most studied and famous bee venom-derived peptide. It inhibits different cancer cells *in vitro*, including leukemic, lung tumor, astrocytoma, glioma, squamous carcinoma,ovarian carcinoma, hepatocellular carcinoma, renal cancer cells, prostate cancer and osteosarcoma.[80] Unfortunately this peptide is toxic to both normal and cancer cells. mastoparan is 14-amino acid cationic peptide isolated from *Vespula lewisii* venom that has shown *in vitro* anticancer activity.[81]

Table 5. *The Anticancer Mechanisms of Some Venomous Peptides and Indirectly Derived Drug*[85]

Target	The Major Mechanisms of Action	Molecular Target	Drug	Drug Class	Indications	Clinical Phase
Ion Channels	The proliferationand invasion of cancer cells	Chloride (Cl^-) channels: CLC3	131I-TM601 (131I-CTX)	Peptide (36aa)	Gliomas	Phase III
			BLZ-100 (ICG-CTX)	Peptide (36aa)	Gliomas tumor marker forsurgery	Phase I
		Sodium (Na+) channels	AGAP	Peptide (66aa)	Colon cancer cells, Malignant glioma cells	Preclinical studies
		Potassium (K+) channels: KV11.1(hERG)	Ergtoxin	peptide (42-62aa)	Ovarian cancer cells	Preclinical studies
		Transient receptor potential(TRP) channels: TRPV6	SOR-C13	peptide(13aa)	Solid tumors with overexpressing the TRPV6 ion channel	Phase I
Integrins	The invasion, migration, angiogenesis, and metastasis of cancer cells	$\alpha_v\beta_3$, $\alpha_v\beta_5$	Cilengitide	Peptidomimetic (5aa)	1 Glioblastoma with methylated MGMT promoter	1 PhaseIII
					2 Glioblastoma with unmethylated MGMTpromoter	2 PhaseII
					3 NSCLC	3 PhaseII
		$\alpha_5\beta_1$	ATN-161	Peptidomimetic	Malignant Glioma	Phase II
		Five integrin receptors ($\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $_v\beta_6$,$\alpha_5\beta_1$)	GLPG0187	Peptidomimetic	Bone metastasis in metastatic breast cancer	Phase I
		$\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$	Vicrostatin	Peptide(69aa)	Ovarian cancer, Gliomas	Preclinical studies
G protein-coupled receptor	The metastasis of cancer cells	Gastrin-releasing peptide receptor	BAY86-7548	Peptide (14aa)	Prostate cancer imaging	Phase II/III
Membrane molecules	The disruption of cancer cell membrane	Sialic acid-rich glycoproteins, PS and PC, heparansulfate	1 MP1	1 Peptide (14aa)	1 Human leukemic Jurkatcells	Preclinical studies
			2 Melittin	2 Peptide (26aa)	2 Human renal cancer, lung cancer, liver cancer,etc.	
			3 Mastoparan	3 Peptide (14aa)	3 Pancreatic cancer cells	
		Phospholipids	Hemilipin	heterodime	HUVECs and HPAECs	Preclinical studies

Most snake venoms are a mixture of several proteins, peptides, toxins, enzymes and non-protein components.[82] Bioactive peptides from snake venoms have significantly contributed to the treatment of many humandiseases, and some of them may selectively target cancer cell membranes, affecting the proliferation of cancer cells.[83] For example, crotamine, a polypeptide of 42 amino acids isolated from South American rattle snake venom; cathelicidin-BF (BF-30) is a cathelicidin-like polypeptide of 30 amino acids and a natural antibacterial peptide extracted from the venom of the snake *Bungarus fasciatus*; purified L-amino acid oxidases from *Bothrops leucurus* which is toxic to cancer cell.[84]

CONCLUSION

This review aims to boost the use of natural product arising from their anticancer activities. Natural product proven to have efficacy asan anticancer activity already. The mechanism of action of many products has been identified and other still under investigation. Overall, natural product research is a vigorous tool to discover novel biologically active components with unique mechanisms of action. Given the diversity of nature, it is sensible to indicate that chemical leads can be produced that are able to interact with most therapeutic targets. As such, new and efficacious drugs can be developed by way of safety treatment of the cancer diseases and get rid of it.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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CANCER STUDIES AND MOLECULAR MEDICINE

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Case Series

Oligo-Metastatic Carcinoma Cervix: A Few Do Extremely Well

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ABSTRACT

The consequence of metastatic carcinoma cervix is regarded to be poor. We present three patients with carcinoma cervix who relapsed in the paraaortic nodes, lungs and vertebra. All of them had oligometastatic disease and were treated with chemotherapy to the lung lesion and radiotherapy to the bone lesion and paraaortic nodes. After more than 5-years they are disease free and on regular follow-up.

Keywords
Carcinoma; Cervix; Oligometastasis; Paraaortic lymph nodes; Chemoradiation; Chemotherapy.

INTRODUCTION

Generally recurrent and metastatic cancer cervix consequences are regarded to be poor. However, some patients with limited metastasis may progress slowly. The data on oligometastatic disease in cervix is limited and hereby we present 3 cases who survived more than 5-years after developing metastasis.

CASE 1

A 40-year-old premenopausal, multiparous lady, was evaluated for intermenstrual bleeding in 2001. Per vaginum examination revealed a 1.5×1.5 cm proliferative growth in the posterior lip of cervix with free fornices, clinically Stage-IB1 disease based on the International Federation of Gynecology and Obstetrics (FIGO) staging system.

Punch biopsy from the lesion showed features of keratinising squamous cell carcinoma. Ultrasound abdomen revealed a mass lesion in the uterine cervix. She underwent radical hysterectomy with bilateral pelvic lymph node dissection. Histopathology was reported as squamous cell carcinoma of cervix infiltrating more than two-third of the thickness, with no significant pathology in the vagina, parametrium, ovaries, fallopian tubes and endometrium. Her post-operative period was uneventful and she was kept on follow-up

In 2004 she presented with complaints of lower backache. She was evaluated with a bone scan which showed increased uptake in the L3 vertebra and sacrum. There was no evidence of local recurrence. She was treated with palliative radiation 20 Gray in 5 fractions, to the lumbar spine and sacrum. She was kept on follow-up with clinical evaluation was performed every 6-months for the first 3-years and then yearly afterwards. A bone scan, performed in 2009 showed no evidence of metastasis. She is currently well and alive in 2019.

CASE 2

A 52-year-old multiparous lady was diagnosed at another centre with non-keratinising squamous cell carcinoma of cervix stage IIA in 2004. She was treated with concurrent chemoradiation, 40 gray in 20 fractions as external beam radiation to the pelvis along with concurrent cisplatin, followed by vaginal brachytherapy. She was on regular follow-up.

In 2008 she presented with complaints of persistent lower backache. She was evaluated with a computed tomography (CT) scan at our centre which showed a destructive lesion with paraspinal soft tissue component involving L5, S1 segment of the

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vertebral body. Multiple enlarged nodes with central hypodensity were noted in relation to the aortic bifurcation with infiltration to the right ureter and right psoas muscle causing right hydroureteronephrosis. A bone scan was performed which showed increased uptake in the L4, L5 vertebra and the upper plate of sacrum. Fine needle aspiration cytology (FNAC) from the vertebral lesion showed features of squamous cell carcinoma, suggesting metastasis from the carcinoma cervix.

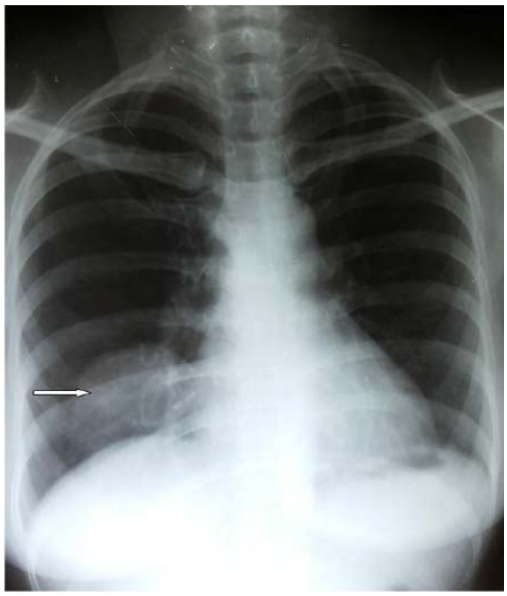
She received chemotherapy (paclitaxel and cisplatin) upto 6 cycles followed by radiotherapy to para aortic nodes and lumbar spine (35 gray in 4-weeks was delivered in view of overlap with old radiotherapy fields). She is on regular follow-up with clinical examination, X-rays of the spine and abdominal ultrasound performed every six-months for the first 3-years and then yearly afterwards. She had no significant symptoms in January 2019.

CASE 3

A 55-years-old postmenpausal, multiparous lady was diagnosed with non-keratinising squamous cell carcinoma cervix, clinically stage IB. She underwent Wertheim's hysterectomy and bilateral lymph node dissection (bilateral obturator and pelvic nodes) at another centre. Histopathology revealed squamous cell carcinoma cervix, large cell non-keratinising type with infiltration to isthmial region and bilateral lymph node metastasis.

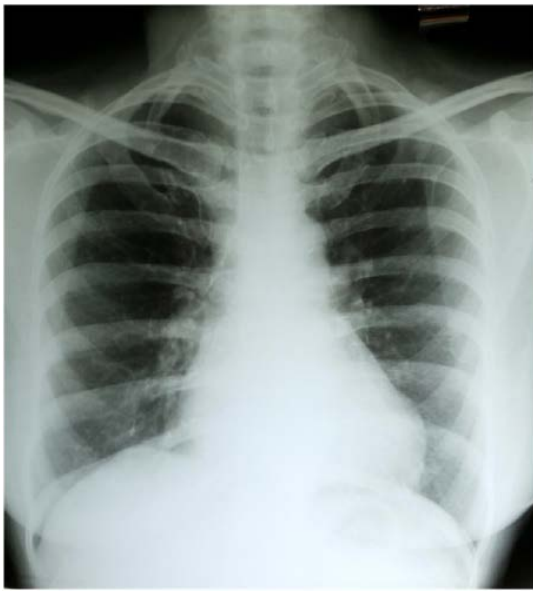
At our centre she received adjuvant chemoradiation, 45 gray in/25 fractions as external beam radiation to the pelvis along with weekly Cisplatin 40 mg/m^2 followed by two sittings of vaginal vault brachytherapy, 3 gray to point A. She was kept on regular follow-up.

Figure 1. *Solitary Metastasis from Carcinoma Cervix in Right Lung*



In November 2005 she complained of of cough. CT chest revealed a 5×3.2 cm mass lesion in the right lower lobe. CT guided biopsy from the lesion favoured squamous cell carcinoma. There was no evidence of local recurrence. She received chemotherapy with cisplatin and 5 fluorouracil with a view to consider surgical treatment after 3 cycles of chemotherapy. But achieved complete response with chemotherapy. Post chemotherapy scans showed no evidence of disease and surgery was not done. Her chest X-rays, before and after chemotherapy are shown in Figures 1 and 2. She is on regular follow-up with clinical examination and chest X-rays performed every 6-months for the first 3- years and then yearly afterwards. She is aymptomatic since January 2019.

Figure 2. *Complete Disappearance of Lung Lesion after Chemotherapy*



DISCUSSION AND CONCLUSION

The term oligometastasis introduced in 1995[1] represents an intermediate state between localized disease and widespread metastasis.[2] The implies is that oligometastasis can be cured with metastasis directed therapy. It is the state in which the patient shows relapse in a limited number of distant sites.[3] Aggressive therapy to metastatic tumors may downsize tumor and the remaining cells may be more sensitive to chemotherapy.[4] For a tumor cell to colonize a distant organ, genetic and epigenetic changes in tumor expression are required, to enable the tumor cells to overcome boundaries, survive in circulation, evade immune system, and colonize distant organs.[5] Gupta and massague have framed the genes important to each of the steps of metastasis and characterized these into three categories-initiator genes, progression and virulence genes.[6]

Oligometastasis or oligorecurrence in distant lymph nodes have been reported in carcinoma cervix due to its spread through the lymphatic route rather than the hematogenous route.[7] The first site of nodal metastasis is usually the paraaortic lymph nodes (PALN). The survival in patients with paraaortic lymph node metastases is considered to be dismal. Its incidence of de-

tection after treatment of primary carcinoma cervix is 1.7% to 12%.[8] Grigsby et al[9] described the outcomes for 20 patients with PALN metastases treated between 1959 and 1986 using conventional radiotherapy with a median dose of 46.4 gray. The survival rate in this study was dismal. All patients died within 2-years after isolated PALN recurrence. Chou et al[10] reported on 19 patients with isolated PALN metastases. Fourteen of the patients underwent cisplatin-based concurrent chemoradiotherapy (CCRT), 4 underwent chemotherapy alone, and 1 underwent rhabdoid tumor (RT) alone. The radiation dose was 45 gray. The 5-year overall survival (OS) rate was 31%, and asymptomatic patients who received CCRT were the only long-term survivors, with a 50% 5-year survival rate.

The frequency of intra thoracic metastasis from carcinoma cervix is approximately 10%[11] while the frequency of bone metastasis from carcinoma cervix is 8.3%.[12] The most frequent site of metastasis was represented by the spine (54.2%) and the pelvis (24.6%).[13] Metastatic spread to the bone may occur both by direct extension of the pelvic tumour to the bone, as from soft tissue metastasis outside the pelvis, and least commonly by haematogenous route.

Many studies have reported survival outcomes of carcinoma cervix patients with paraaortic lymph node metastasis but data regarding oligometastasis to lung and skeletal system are scant.

In this case series, we have reported outcomes of a few patients with oligometastases to lungs, bone and paraaortic lymph nodes. There were no patient-related or disease-related factors which could account for the prolonged survival. There were no clinical or pathological differences between these cases and other high grade cases. It has been demonstrated that, patients with carcinoma cervix with oligometastasis have a long survival when treated with a radical intent.

CONSENT

The authors have received written informed consent from the patient.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

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Opinion

The Phenotype Landscape of Cancer in the Genotype Era

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Some tumor types such as testicular cancer, leukemia and lymphomas, have their origin in cells that comport a common embryonic origin. This phenomenon was "unconsciously" the roadmap to treat and cure them by classic chemotherapy. The cells are very chemosensitive indeed as they are "immature" in origin.

Chemotherapy has also positively impacted the overall survival of some pediatric and adolescent cancers (leukemias, lymphomas, bone tumors) and in many other adult ones such as breast, ovarian, head and neck and colorectal cancers. Despite everything, we also face other difficult to treat cancers which remain chemo resistant (lung, melanomas, pancreatic, biliary tract cancers as the most representative ones).

As basic and translational cancer researches evolve, these tumor-types have turned into a significant focal point of intrigue. So, what to do in order to work-out this cancer resistance dilemma? Targeting some tumor genetic mutations such as those of the tyrosine kinase types, oncogene- driven growth factor receptors and some immune molecules such as those of the programmed-death pathway (PD-1 and PD-L1) and the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) axis, enable us to battle the malignant growth.

Regarding these aforementioned cellular pathways, drugs which are of small molecules, target the first two druggable ones and treat lung, renal and malignant melanoma. The third one, "the mammoths": monoclonal antibodies (MABs), can disrupt angiogenic pathways, block some oncogenic drivers (such as RAS) and mainly deblock the lazy T-lymphocytes by allowing them to "eat tumors" (checkpoint inhibitors to treat melanoma, lung, squamous head and neck tumors). This last one, became the treatment of cancer by the inhibition of negative immune regulation for which the researchers have been granted Nobel prize.[1]

But, all the before-mentioned is a "partial win" in the treatment of cancers. There will be still continued exploring "for more and better" in the above-targeted therapy.

The answer for therapy failures is related to the old concept of tumor heterogeneity. Imagine a view with the light microscope, where the tumor sample even seeming homogeneous, is composed of "cells of different imaginable colors". Tumor cell phenotype heterogeneity is the main cause of secondary resistance and also of progression in the already mentioned chemosensitive tumors, and one of the main causes of the non-tumor responses in the less *de novo* chemosensitive ones such as lung cancer.

Nowadays, tumor heterogeneity explains why therapy targeted to a driver gene or protein in a tumor is met with disease progression, the disease at some point now it has a new driver gene in some cells, or more easily the emerging presence of a new clonal cell population. So, is the answer we need to explore for new biomarkers? Yes or no.

Tumor heterogeneity is something more than this, it´s mainly the cellular dynamics between the different tumor clones that lead to resistance. These dynamics are essential to the phenotypic landscape of the cancer cell populations. Tumor cells have a "great talk" between them (growth factors signals). They have cooperation within the different clones: two clones evolve complementary traits. Even though each clone is not self-sufficient, their cooperation results in malignancy.

They have competition for oxygen and substrates, and also interactions with the mesenchyme in the case of epithelial tumors. In this sense, clonal lineages with similar fitness may coexist and compete within a tumor, a phenomenon known as clonal interference. This adds a higher complexity to the cancer-disease

process. In origin, cancer is a one-cell-disease but, it is a tissue disrupted-related disease during the rest of its whole lifecycle. With the old, current and novel drug-tools, we must learn "how to treat the cancer phenotype in a better way." In the early days of chemo, in some way all the aforementioned have been taken into account: tumor cellular kinetics explained by the Goldie-Coldman model measures genetic instability to the probability of a tumor containing drug-resistant clones. This works as a framework for understanding some types of tumor cell kinetics and posterior chemo responses and resistance in them.

My opinion here is to highlight the tumor heterogeneity with its clonal dynamics as a whole: the Cancer Phenotype.

Cancer can also be considered as an evolutive Darwinian process, where the most adaptable cancer cells within a heterogeneous population survives, proliferate and invade other organ-sites. Phenotype plasticity is an enabler of tumor progression and therapy resistance. Within it, is tumor heterogeneity what makes malignancies as the "masters of evasion".

On the other hand the tumor immunophysiology, the scenario is even more complex in its phenotypic assessment, with different cancer-immune phenotypes, for e.g., inflamed-tumor, immune-excluded tumor and immune-desert tumor.

Each is associated with specific underlying biological mechanisms that may prevent the host´s immune response from eradicating the tumor. Similarly, an immune-desert phenotype can be the result of immunological ignorance, the induction of tolerance or a lack of appropriate T-cell priming or activation. Immune-excluded tumors may reflect a specific chemokine state, the presence of particular vascular factors or barriers, or specific stromal-based inhibition. Finally, inflamed tumors can demonstrate infiltration by a number of subtypes of immune cells (T-cells, myeloid-derived suppressor cells, suppressor B-cells and cancer-associated fibroblasts such as in Melanoma).

Finally, for some difficult to treat cancer scenarios, where the disease has a progression over progressions or are directly refractories ones, the medical therapy to be utilized are in an adaptive or metronomic manner, directed to some tumor cell populations while keeping up calm on others with the lesser growth (e.g., mesothelioma, pancreatic carcinomas, biliary tract cancers mainly).

Some cancers can be considered as chronic diseases. As a physician, the "*primum non-nocere*" concept is essential: "*to treat the patient, not just the cancer*".

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