Molecular Targets For Nutrients Involved With Cancer Prevention

1.1. INTRODUCTION

Dietary nutrients can influence cancer risk by inhibiting or enhancing
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Chapter-I

carcinogenesis through diverse mechanisms of action. The identification and elucidation of their sites of action have been a focus of nutrition and cancer research for more than four decades.

Transforming nutrition and cancer research from a predominantly observational to a molecular approach offers exciting opportunities for truly identifying those who will and will not benefit from dietary intervention strategies. There is evidence that genetic polymorphisms can influence the dynamics between nutrients and molecular targets and, thus, contribute to variation in response among individuals. Because many molecular targets will likely be identified, it may be necessary to credential nutrients, that is, to determine which specific nutrient-related genetic and epigenetic changes bring about phenotypic changes, to establish which interactions are the most important and under what circumstances.

Vitamin D, calcium, folate, selenium, genistein and resveratrol are highlighted, because they represent specific classes of nutrients and illustrate the need to credential various nutrients to understand their physiological significance in cancer prevention. As the science of nutrition unfolds, a clearer understanding will emerge about how nutrients can modulate cancer risk through molecular interactions and how foods might be changed by agronomic approaches and biotechnology. Undeniably, embracing new genomic technologies offers exciting opportunities for advances in the broad area of nutrition, especially those related to cancer prevention.

Della Penna coined the term nutritional genomics to describe work at the interface of plant biochemistry, genomics, and human nutrition aimed at understanding and manipulating nutrient reactions and interactions at the molecular or genomic level. For purposes of this work, nutritional genomics refers to the study of any genetic or epigenetic interaction with a nutrient that leads to phenotypic changes. Genetic interactions involve direct alteration of the DNA coding sequence, whereas epigenetic changes are mechanisms exclusive of direct modification or damage to DNA. The basis for nutritional genomics arises from rather compelling evidence that a variety of nutrients influence genetic and epigenetic processes and gene-regulated metabolic pathways through interactions.
with specific molecular targets. These molecular targets may be individual genes, molecules that result from gene expression are otherwise affected by gene expression, or any other molecular events that are relevant to the process of carcinogenesis. (John A. Milner et al. 2001).

1.1. A. INTRODUCTION TO TUMOUR

A tumour or cancer may be defined as an abnormal lump or mass of tissue, the growth of which exceeds, and is uncoordinated with that of the normal tissue, continuing after the stimuli that initiated and forms discrete masses. Tumors vary widely in their growth rates. The most important classification of tumour is that of benign or malignant.

Benign tumor cells remain at the site of origin, forming a cell mass. When growing in a solid tissue, they usually become enclosed in a layer of fibrous material, the capsule, formed by the surrounding tissues. Benign tumours rarely kill, unless they press on a vital structure or secrete abnormal amount of hormones.

Most fatal tumours are malignant or cancerous. Unlike benign tumors, the cells of malignant tumours invade locally, and often also pass through the blood steam and lymphatic system to form secondary tumours (metastases) at other sites. The rates of growth and metastasis formation differ from tumour to tumour. Tumour is also classified according to the tissues from which they arose, e.g. carcinomas such as adenocarcinoma arise from epithelial cells and sarcomas from connective tissue or muscle. (Gower J.D. et.al. 1988).

Carcinogenesis

The process of conversion of a normal cell to the malignant state is called carcinogenesis, and agents that induce it are called carcinogens. Carcinogenesis is a complicated, multi-stage process; essentially, a small population of abnormal cells is generated and then increases in abnormality as a result of a series of mutations and changes in the patterns of gene expression.(Gower J.D. et.al. 1988).

Table 1.1: The Stages Of Carcinogenesis

<table>
<thead>
<tr>
<th>Stages</th>
<th>Description</th>
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### Initiation
- Cellular phenomenon characterized by genetic changes that are irreversible.
- Example of initiators includes agents that react directly with DNA (endogenous or xenobiotic) and ionizing radiation.
- Single initiated cells are not morphologically recognizable may have subtle phenotypic changes after multiplication depending on organ.

### Promotion
- Reversible process of gene activation
- Often the result of action of xenobiotics or endogenous substance involve entire tissue may produce benign tumour from initiated cells.
- Examples of promoters include phorbol esters in the mouse skin, saccharin in the urinary bladder and phenobarbital in the liver.
- May have at least two stages, early stage is reversible and late is irreversible.

### Progression
- Conversion of benign to malignant tumours usually accompanied by more rapid growth, invasiveness, metastasis, increased genetic instability.
- May be associated with further irreversible genetic change like loss of a tumour-suppressor gene.

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### Reactive Nitrogen Species and Nitrosamines
Nitrous acid and ONOO- can deaminate DNA bases and are thus mutagenic and potentially carcinogenic. Reaction of secondary or tertiary amines with nitrosating agents produces N-nitrosamines (in the case of a secondary amines, for example, N-H is replaced by N-N=O). Nitrosamines can be formed in the stomach by reaction of dietary amines with salivary (or food-derived) nitrites at the low gastric pH, and they can be generated sites of inflammation when RNS from activated phagocytes react with amines (Orshima, H. et al., 1994). Chronic infections and inflammatory process as cancer risk factors are possible role of NO in carcinogenesis.

Nitrosamines are found in human faeces (levels increase on a high meat diet) of tobacco smoke is a rich source of nitrosamines, some of which are tobacco
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Nitrosamines require metabolic activation (involving cytochromes P450) to agents that can methylate guanine. Ascorbic acid and possibly various plant phenolics (such as flavonoids), may be important scavengers of nitrosamines in vivo. (Wagner DA et.al., 1985).

Etiology of cancer

It was described by Sager (1983) that the cause of cancer as a multistage genetic process. The stages include: 1. Initial DNA damage 2. Chromosome breakdown and rearrangement gene replication and 3. Selection of successfully growing mutant cells.

The initial changes in cellular DNA can be caused by a variety of carcinogens, including radiation, chemicals, viruses, and unknown agents. This leads to faulty growth control and loss of chromosome stability. The chromosome breakage and rearrangement occur in several continuous phases after the initiation of cell division. This is later manifested in terms of aberrant chromosomal transpositions, which lead to genomic rearrangements.

The changes in the DNA and chromosomes result in a new pattern of gene expression, creating a new phenotype, in which previously quiescent genes are now expressed, previously expressed genes are now quiescent, or there is over expression of certain key genes. It is now believed that the earliest changes in gene expression that can lead to a transformed cell occur in genes that normally regulate cell growth and cell death. These newly expressed or suppressed regulatory genes are known as oncogenes.

As cancer cells multiply, there may be additional phenotypic changes in now unstable genetic material. As a result, a process of natural selection allows the most “successful” cancer cell to proliferate the most and to dominate the cancer mass. As the environment surrounding the cancer changes, such as occurs as a result of therapy, the selection process continues.

Diversity of Cancer Cells

There is a broad range of possible combinations of gene expression in the human cell. It varies from normal cells to the most atypical cancer cells. As a
result of the genetic changes described above, cancer cells develop new combinations of gene expression and therefore new phenotypes. The phenotypic variation occurs not only from cancer cells to normal cells or from cancer type to cancer type, but also within particular cancer types and even within a single tumor. For example, in patients with cancer of the breast there is heterogeneity of genes expressed by various cells; that is, not all cells express the same genes. An example is the heterogeneous expression in breast tumors of the gene for the estrogen receptor.

Variable gene expression leads to biological and biochemical diversity of cancer cells. Consequently all cancer cells of the same type or even a single cancer do not necessarily elaborate various tumor-specific markers over time. This fact is clinically very important in an investigator’s determination of which analyses to follow in monitoring patients with known malignancy. The cellular diversity within a single tumor malignancy that is a cancer’s clinical manifestation, such as a tumor’s response to therapy, may change with time.

Clinical Manifestations

The clinical manifestations of cancer vary widely, depending on the type of tumor, the tissue affected, and the stage of tumor development. For example, obstruction, hemoptysis, and bloody stools manifest cancer of the gastrointestinal tract. Cancer of the lung is manifested by hypoxia, chest pain, and often various neurological symptoms. The clinical manifestations are related to the physiological function of the organ with the primary cancer and the effect of the cancer on other organs as well. For example, cancer of an endocrine gland can result in production of excess hormone with many systemic hormonal effects. New symptoms are seen with the spread (metastasis) of the cancer cells to other organs. Cancer spreads through the lymphatic system and the blood stream, resulting in liver, bone and pulmonary metastases.

Time as a Factor: Cancer as a Long Term Process

Cancer is a long-term process and progresses through four obligatory phases: an induction phase, an in situ phase, an invasion phase, and a dissemination phase. During the induction phase, which can last up to 30 years or
more, the cells are exposed to one or more carcinogens. These environmental carcinogens may include radiation or various toxins. It has been estimated that approximately three-fourths of all human cancers may be caused by these environmental factors.

It is now believed that a period of many years after exposure may be necessary before a carcinogen is able to have its effect on the host. The histological changes begin with severe dysphasia, eventually leading to cancer. Obviously, not everyone who is exposed to the same carcinogen will develop cancer. Additional factors that play a role in deciding that may get cancer include individual (genetic) or tissue susceptibility, the presence of other carcinogens may act, the duration of exposure and of course, the nature, amount and concentration of the carcinogen under question. Often the time between the induction phase and the clinically apparent cancer can be as long as 20 years.

After induction there is the \textit{in situ} phase. The phase represents that time during which the transformed cell actually develops into a cancer but the cancer remains localized in the original site and does not invade other tissues. Colonel selection for those cancer cells that grow most successfully occurs during this phase.

The third phase is called the invasion phase. During this phase the malignant cells multiply and invade into the deeper tissues through the basement membrane, thereby gaining access to blood vessels and lymphatic channels.

The fourth stage is the dissemination phase. During this phase, which lasts 1 to 5 years, the invading cancer spreads to various parts of the body distant from the site of origin, often through the blood distant from the site of origin, often through the blood and lymphatic systems. One factor limiting tumor growth during this phase is the formation of a new blood supply. This process, termed angiogenesis is regulated by the presence of vascular endothelial growth factor (VEGF).

Early detection of cancer, before metastasis spread, is critical to improve success in treating the disease. In fact, it would be ideal to detect cancer during the induction phase; Unfortunately, however, this is impossible because prior to the \textit{in situ} phase, an investigator cannot be certain whether cancer will actually
develop in the individual. The next best approach, then, is to detect the cancer during the *in situ* phase. This has been done with great success in patients with cancer of the cervix, in which the Pap (Papanicolaou) smear technique has been of great benefit. When *in situ* cancer of cervix is detected, the prognosis is excellent. Most cancers are detected during the invasion phase. If dissemination has not yet occurred, the prognosis is reasonable. Detection of local spreading with or without involvement of the lymph nodes often leads to a cure. However, if dissemination has already occurred, the prognosis is very poor.

**Invasion by Cancer Cells of Surrounding Tissue**

Several factors play a role in determining the cancer’s ability to invade the surrounding tissue. Such factors include increased motility of the cells, increased pressure within the tumor caused by active multiplication of the cells, elaboration by the cancer of lyric substances, lack of intercellular bridges found between all normal cells, decreased cohesiveness between cells, and eventual spread of the tumor cells to the regional lymph nodes. However, when the metastases are still microscopic (micro metastases), the clinician’s ability to detect them is very poor. It has been estimated that approximately half the patients who appear to be clinically free of metastases do in fact have unrecognized distant micro metastases at the time of initial diagnosis and treatment.

**Change in Cell Division**

Cancer is often manifested by a change in cellular division rate. Although most cancers are associated with an increased rate of cell division, there are examples, such as nephroma, in which this is not always the case.

**Dedifferentiation of Cells**

A common phenomenon of cancer is dedifferentiation, in which cells go from a more specific cell type to a more general cell type by the process of clonal selection. Thus it is not uncommon for cancer cells to synthesize various compounds that are normally present only in the embryonic or fetal stage. On the other hand, as cells dedifferentiate, they may lose certain specific cellular properties such as receptor activity or enzyme activity. These phenotypic changes
can be used as prognostic indicators.

Chromosomal Changes in Cancer

Chromosomal changes in cancer have been extensively studied in patients which leukemia, and various types of leukemia are often confirmed on the basis of these chromosomal changes.

A stepwise model of colorectal tumor genesis involving gene mutations and chromosome changes was developed in 1990 and has since been validated. Subsequent work has discovered additional genetic events and specific molecular pathways affected by these mutations. This model, reviewed by Chung summarized here, may serve as a general description for the development of cancer cells. In this model the following sequential steps must occur for the development of colorectal cancer.

1. Mutations in the adenomatous polyposis coli (APC) tumor suppressor gene occur early in the development of polyps.
2. Oncogenic K-ras mutations arise during the adenomatous stage.
3. Mutations of TP53 and deletions on chromosome 18q coincide with transition to malignancy.

The APC gene encodes a protein which, when mutated, results in either familial adenomatous polyposis (FAP) or Gardner’s syndrome; it also plays a critical role in sporadic colon cancer. The APC tumor-suppressor gene is mutated in more than 70% of all colorectal cancers. The mutated APC protein causes a disruption of the APC/beta-catenin, which trans-locate to the nucleus, where it activated several oncogenes.

The DNA mismatch repair pathway. Mutations in five different genes have been identified with the Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome. Each of the genes encodes a protein involved in DNA mismatch repair; the enzymatic process which corrects base pair mismatched arises during DNA replication. These gene defects presumably cause tumor development as a result of widespread mutations that are unable to be repaired.

Factors identified in promotion of metastasis. The process of metastasis requires a number of biological and chemical alterations to the cancer cells.
This includes reduced expression of cellular adhesion molecules (cadherins) and increased degradation of the extra cellular matrix components by metalloproteases and serine proteases, which allow the cells to detach from one another and move from the primary tumor mass. Finally, interactions among tumor cells and cells of the surrounding environment, mediated by cytokines and growth factors lead to the establishment of metastases.

1.1.B. ROLES OF LABORATORY TESTS

Laboratory tests can serve four major functions in the field of neoplasia: detection or screening, confirmation, classification (staging), and monitoring.

Detection

The table lists several screening tests for early detection of cancer. The quality of a screening test is usually expressed by its clinical sensitivity and specificity. The observations from the screening tests are divided into negative and positive results. Each person examined are classified as either a diseased or a no diseased person.

A rigid classification of test results into positive and negative results may sometimes be too simplistic. Outcomes of screening tests can usually be ordered from very negative to very positive. The latter approach allows for a more sophisticated test interpretation in actual screening programs. For example, patients whose results are not negative but also are not alarming enough to justify immediate diagnostic action can be scheduled for earlier repeat screenings. Another example is a stepwise screening policy in which only individuals with positive results at the first screening test are subject to further diagnostic testing.

Sometimes the use of more than one screening test may seem advantageous. However, assessment of the sensitivity and specificity of a combination of screening tests based on data available for the individual tests in complicated by the fact that usually the tests are not independent in a statistical sense. In general, it is more effective to combine two tests that are complementary (i.e., directed at different anatomical or biochemical features of the tumor) than to
combine tests directed at the same types of features. Examples of complementary tests include sputum cytological examination and chest x-ray examination for lung cancer screening. On the other hand, palpation and mammography in breast cancer screening are examples of two related tests. They both detect tumors largely on the basis of size.

**Table 1.2: Screening Test for Early Detection of Cancer**

<table>
<thead>
<tr>
<th>Site</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Cytological analysis of urine</td>
</tr>
<tr>
<td>Breast</td>
<td>Mammography, physical examination and Self-examination</td>
</tr>
<tr>
<td>Cervix</td>
<td>Papanicolaou smear and pelvic examination</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>Testing stool for occult blood and Sigmoidoscopy</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>Physical examination and roentgenography</td>
</tr>
<tr>
<td>Lung</td>
<td>X-ray, cytological analysis of sputum</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>Visual examination</td>
</tr>
<tr>
<td>Prostate</td>
<td>Prostate-specific antigen, digital palpation by rectum</td>
</tr>
<tr>
<td>Skin</td>
<td>Visual inspection</td>
</tr>
<tr>
<td>Stomach</td>
<td>Photofluorography, saline wash and cytological examination of gastric contents and examination of stool for occult blood.</td>
</tr>
</tbody>
</table>

**Confirmation**

Additional tests are used to confirm the suspicion of cancer based on clinical symptoms or signs. Tests that tend to confirm the presence of a cancer includes, for example bone marrow examination for leukemia, urinary catecholamine for pheochromocytoma and alpha-fetoprotein for testicular cancer.

**Classification and staging**

Classification of tumors is used to describe the degree of tumor differentiation. Tumors are classified as well differentiated, moderately well differentiated, and poorly differentiated. Poorly differentiated tumors are more aggressive and have a poorer prognosis. Surgical pathologists have developed various staging approaches based on the size and extent of invasion of surrounding tissues by the tumor, the number of cancer cell-positive lymph nodes,
and the presence or absence of metastases. This has been called the TNM (tumor, nodes, and metastases) system. The purpose of such staging is to give reasonable estimates of prognosis (i.e., recurrence of cancer), appropriate response to therapy, or the likely course of the disease. In addition to staging based on gross or microscopic pathological data, it would be of great value to have biochemical tests that could classify cancers appropriately. It has been suggested that an elevated serum prostatic acid phosphatase level can indicate the presence of metastatic prostate cancer.

**Monitoring**

The most important function of laboratory tests in cancer is that of monitoring the course of the disease or its response to therapy. Winkel et al have developed various strategies to monitor patients known to have breast cancer, addressing the problem of predicting on the basis of sequential values whether a patient would have recurrence of this disease. Other approaches were advanced in the mid-1980s to monitor patients on the basis of carcinoembryonic antigen (CEA) in colon cancer, prostate-specific antigen (PSA) for Prostate cancer and others. An increased CEA or PSA value may indicate a need to modify treatment. It is assumed that the CEA or PSA-producing tumor has reached clinical proportion when the serum values for PSA or CEA reach a certain threshold. (Lawrence A. Kaplan et al. 2003).

### 1.1. C. TUMOR MARKERS

Coombes and Neville have suggested that the ideal tumor marker should fulfill the following criteria:

1. Should be easy and inexpensive to measure in readily available body fluids.
2. Should be specific to the tumor studied and commonly associated with it.
3. Should have a stoichiometric relationship between plasma level of the marker and tumor mass.
4. Should have an abnormal plasma level, urine level, or both, in the presence of micrometastases, that is, at a stage at which no clinical or presently
available diagnostic methods reveal their presence.

5. Should have plasma level, urine levels, or both, that are stable and not subject to wild fluctuations.

   If present in the plasma of healthy individuals, exist at a much lower concentration than that found in association with all stages of cancer.

   Obviously, much additional research must be done before such ideal tumor markers can be found. However, it is important to recognize that the evaluation of an ideal tumor marker should relate to the clinical setting. To this end, it has been suggested that all tumor markers should also comply with the following major criteria.

1. They should prognosticate a higher or lower risk for eventual development of recurrence.

2. They should change as the current status of the tumor changes over time.

3. They should precede and predict recurrences before they are clinically detectable.

   In addition, if a tumor marker is to be used to detect very early stages of cancer, a treatment for the cancer must be available. It might be unethical to detect cancers for which no effective treatment is available.

   All tumor markers should be analyzed both according to the criteria that Coombes and Neville have presented and according to the considerations just mentioned. For a tumor marker to be of some value, it must give information beyond that readily seen on the basis of physical examination or history, and it must give this information with a reasonably long lead time to allow appropriate therapy to be given in a timely manner. Lead-time is the time elapsed between the points when a test result is positive and the time when the disease is clinically evident or advanced.

**Ethics of Testing**

Even when a tumor marker can be used to detect the presence of disease, it may not always be beneficial to use it. The reason is that prostate cancer is usually slow growing and in most cases it is more likely that a patient will die from some
other cause, rather than from the prostate cancer. The standard treatments for early prostate cancer (brachytherapy, external radiation, and surgical intervention, all with or without chemotherapy) can result in unwanted side effects, including impotence, incontinence, and the need for colostomy. In addition, a significant number of patients can die from the treatments themselves.

Classification of Tumor Markers

Oncofetal Proteins
- Carcinoembryonic antigen (CEA)
- Alpha-fetoprotein (AFP)
- Human chorionic gonadotropin (hCG)
- SCC (Squamous Cell Carcinoma) antigen

Mucin Glycoproteins (Carbohydrate Antigen)
- CA-125
- CA-19-9
- CA-15-3 and CA-27-29

Enzymes
- Prostate –specific antigen (PSA)

Hormones and Hormone Receptors
- ACTH and all other endocrine hormones
- Breast estrogen and progesterone receptors

Cell Surface Proteins (Other than Receptors)
- Beta2-microglobulin

Cellular Markers
- Oncogenes, such as N-ras
- Suppressor genes, such as p53
- BRCA1 and BRCA2
- C-erB-2 (HER-2) neu

Oncofetal Antigens
Many of the oncofetal antigens are measured in the laboratory by use of solid –phase immunometric assays, employing second antibodies labeled with enzymes, fluorescent or chemiluminescent compounds. These proteins are not recommended for cancer screening.
The tumor markers discussed previously are antigenic proteins with several distinct antigenic sites. Immunoassay characteristics for these markers are based on antibody affinity and antibody specificity, and the specificity of the assay depends on the recognition of one or more of the antigenic determinants. Obviously, antibodies form different reagent vendors will react differently with the antigen. Since these antigens are glycoproteins and the carbohydrate portion of the molecule may differ from patient to patient. Antibodies from different vendors may also react differently from one patient to the next. Therefore, when patients are being monitored, the assay used should be from the same manufacturer during the monitoring period. Otherwise, analytically significant changes may occur during serial monitoring as a result of a change in the source of the tumor marker reagent. If it is necessary to change the vendor source, individual patient parallel studies should be performed in which at least two specimens are analyzed by both methods so that the physician can compare individual patient results.

Carcinoembryonic antigen

CEA is a glycoprotein present in colonic adenocarcinoma and fetal gut; Gold and Freedman first described it. The detection of CEA in various tissues or serum is complicated by the presence in these tissues of CEA cross-reacting antigens.

In general, CEA plasma levels increase with increasing age and smoking. This has prevented the use of CEA levels for the purpose of general screening. The results of screening programs confined to subpopulations with higher-than-average risk of developing cancer have been equally discouraging. Thus, neither the sensitivity nor the specificity of CEA justifies its use for the definitive diagnosis of cancer.

In specific situations, however, CEA has proved to be of diagnostic value. CEA is useful, for example, for the detection of primary colorectal cancer when used in combination with a barium enema and with radio iodide imaging for the detection of carcinoma metastasis to the liver. According to the consensus statement of the National Cancer Institute, only values, five to ten times the upper
normal reference limit in patients with symptoms should be considered strongly suggestive of the presence of cancer. In some cancers, including colorectal and breast cancer, the plasma level of CEA and the frequency of elevated values are positively correlated with the severity of the disease as assessed by clinical staging. Currently, CEA is approved only for monitoring of colorectal cancer.

**Alpha-fetoprotein and human chorionic gonadotropin**

Alpha-fetoprotein (AFP) is an oncofetal glycoprotein. In early embryonic life it is a predominant component of the serum proteins. It is first synthesized by the yolk sac and later by the fetal liver. Later in life it is mainly produced in the liver. AFP was first recognized as a tumor marker by Abele in 1963.

Serum AFP values should be less than 10μg/L in healthy subjects. In being hepatic disorders, moderate elevations (40μg/L) may be seen. Values above 400μg/L are almost always associated with hepatocellular carcinoma, germ cell carcinoma (such as testicular carcinoma), chronic aggressive hepatitis, or sub acute hepatic necrosis. Currently, AFP is approved only for use with testicular carcinoma and hepatocellular carcinoma.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that can be secreted in large amounts by the trophoblastic tissue of tumors of the placenta and the testes. Specific and sensitive assays have revealed that many other cancers can also secrete hCG. However, available data clearly show that hCG determinations are of no value in screening for cancer.

**Mucin Glycoproteins (Carbohydrate Antigen)**

**Carbohydrate antigen-19-9**

Carbohydrate antigen-19-9(CA-19-9) occurs in tissue as a monosialoganglioside and in serum as mucin, a high-molecular-weight, carbohydrate-rich glycoprotein. Results of clinical studies indicate that the CA-19-9 level in serum or plasma of patients with an intra abdominal carcinoma is frequently increased. It is correlated most strikingly with cancer of the pancreas, for which early studies have shown a sensitivity of 90% and a specificity of 85%. CA-19-9 also may be increased with other adnocarcinomas such as lung, gastric,
biliary, and colon.

**Carbohydrate antigen-125**

Serum carbohydrate antigen-125 (CA-125), a glycoprotein antigen, is elevated in the serum of patients with ovarian cancer. Increased concentrations of CA-125 were found in many patients with epithelial ovarian cancer and in ovarian teratoma. Changes in CA-125 concentrations in serum during chemotherapy mirrored the progress of the disease as assessed by clinical and radiological evidence. It should be noted that CA-125 provides no real assistance for diagnosis; however, it does have value as a marker for monitoring responsiveness to chemotherapy.

**Carbohydrate antigen-15-3 and 27-29**

Serum carbohydrate antigen-15-3 (CA-15-3), a glycoprotein antigen, is elevated in the serum of patients with breast cancer. Changes in CA-15-3 concentrations in serum after surgery or during chemotherapy mirrored the progress of the disease as assessed by clinical and radiological evidence. Just as with the CA-125 antigen, CA-15-3 provides no actual diagnostic assistance, but it does have possible value as a marker for monitoring responsiveness to chemotherapy.

**Enzymes**

Schwartz reviewed the use of enzyme tests in the management of patients with cancer. The use of enzyme markers is fraught with difficulties. Not all patients with a particular cancer type have elevations in an enzyme (poor sensitivity); furthermore, many non-cancerous diseases are associated with elevations of many of these enzymes. Thus the most frequent uses of these enzymes are as objective markers to give semi quantitative estimates of response to therapy or as prognostic indicators. However, enzyme markers have generally been replaced by the oncofetal markers and are rarely used today. An exception to this is PSA, which is an extra cellular protease.

Prostate-specific antigen (PSA) exists in serum in several molecular forms,
including a free or non complexed form, and complexes of PSA with serine protease inhibitors alpha-antichymotrypsin (ACT) and alpha$_2$-macroglobulin (which is not detectable with current assays). Total PSA is a combination of all immuno detectable forms in serum, primarily free PSA and PSA-ACT. The complexed form is the predominant form found in serum.

**Hormones and Hormone Receptors**

Both estrogen and progesterone receptor assays are useful in the assessment of the prognosis of patients with breast cancer. These procedures evaluate the relative concentration of receptors for estrogen and progesterone in breast tumor excised during surgery. Individuals who are positive both for estrogen and progesterone receptors tend to have a longer survival time and thus a better prognosis than individuals who are deficient in these receptors.

**Cellular Markers**

**Oncogenes**

There are genes capable of causing cancer. Michael Bishop and Harold Varmus, pioneers in the oncogene research were awarded Nobel Prize in 1989. A definite proof for an oncogene was first demonstrated in Rous sarcoma virus (Cancer arising from epithelial tissue is carcinoma, that from connective tissue is sarcoma). The full virus produces sarcoma in avians but a strain of virus deficient in a particular gene, could not cause the disease. Hence this gene was names as sarcoma gene, abbreviated as src. This clear-cut proof was a little blurred, when it was shown by DNA hybridization technique that the same DNA sequences are available in normal avian cells also. This reveals that normal cells do contain DNA sequences similar to viral oncogenes. To distinguish these two genes, they are denoted as V-src (viral gene) and C-src (cellular gene).

The oncogenes present in normal cells are also called as proto oncogenes. Today, more than 80 human proto-oncogenes are known. They were located on specific chromosomes. Lists of some important c-oncogenes are given here.

| Table 1.3: Some Cellular Oncogenes | 18 |
Present day concept is that proto-oncogenes are important regulatory genes of the cells. In fact, viruses carry these genes accidentally picking them from the host cells during the evolutionary process. The cellular oncogenes produce specific proteins, which are defined to have a role in nucleus, cytoplasm or cell membrane. They may act through different mechanisms. (1) Products of many oncogenes are polypeptide growth factors, e.g. sis produces platelet-derived growth factor (PDGF) released from alpha-granules of platelets. This factor is required for normal wound healing. (2) Some of the products act as receptors for growth factor, e.g. erb-B produced receptor for EGF (epithelial growth factor). They act on key intracellular pathways involved in growth control. Definite molecular events are known to activated by oncogene products, e.g. src product, a membrane-bound enzyme, phosphorylates a specific tyrosine residue, leading to cascade activation of cellular events. Src product also catalyzes phosphorylations of phosphatidylinositol to phosphatidylinositol-4, 5-bisphosphate. This is hydrolyzed to diacylglycerol, which stimulates plasma membrane bound protein kinase C, which in turn phosphorylates a number of proteins, including important
ion pumps. Receptors for EGF, insulin, PDGF, etc. are also activated by src-product protein.

The C-oncogenes generate proteins essential for normal functions as well as for cell division. But the C-oncogenes are under the control of regulatory genes, and expressed only required when virus enters, and extra oncogene is inserted so as to produce continuous expression of the gene leading to uncontrolled cellular activity and malignant transformation.

Proto-oncogene activation has been demonstrated in about 15% of different types of human tumours. Proto-oncogene may be activated by many factors. A few important mechanisms are given below.

**Chromosomal Translocation**

In Burkitt’s lymphoma, translocation of chromosome 8 to 14 with consequent activation of c-myc has been described previously. In chronic myeloid leukemia, deletion of short arm of chromosome 22, called Philadelphia (Ph’) chromosome is seen in 80% cases. In the rest, there is translocation of 9 to 22 leading to activation of c-abl present in chromosome 9. In Non-Hodgkin’s lymphoma, translocation of chromosome 14 to 18 is very common. The breaks on chromosome 18 occur either in major break point region (MBR) or at the minor cluster regions (MCR), both involving the bcl-2 oncogene. The bcl-2 product suppresses programmed cell death (apoptosis) leading to tumor formation.

**Promoter Insertion**

The virus becomes integrated near the c-myc gene. The viral gene acts as an enhancer.

**Point Mutation of Proto Oncogene**

The ras gene produces a protein termed P$_{21}$ (Mol. Wt. 21,000) related to the GTPase that suppresses the activity of adenyl cyclase. Adenyl cyclase has a key role in cellular response to hormones. C-ras oncogene product in human bladder cancer differs solely in a substitution at 12$^{th}$ amino acid of the P$_{21}$. 
GTPase activity is decreased leading to continuous activity of adenyl cyclase because the altered $P_{21}$ is inefficient. (Lawrence A. Kaplan et.al.2003) (Vasudevan D.M et.al.19980).

**Tumor Suppressor Genes** (Antioncogenes or Oncosuppressor Genes)

These are the genes, which normally protect the individual from getting cancer when the gene is deleted or mutates. Cancer results only when both alleles of the RB gene are deleted (homozygous) retinoblastoma.

**Table 1.4: Some Oncosuppressor Genes**

<table>
<thead>
<tr>
<th>Name of the Oncosuppressor</th>
<th>Abbreviation</th>
<th>Located in Chromosome no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma</td>
<td>RB</td>
<td>13</td>
</tr>
<tr>
<td>Wilm’s tumour</td>
<td>WT</td>
<td>11</td>
</tr>
<tr>
<td>Familial adenomatous Polyposis</td>
<td>FAP</td>
<td>5</td>
</tr>
<tr>
<td>Deleted in Colon Cancer</td>
<td>DCC</td>
<td>18</td>
</tr>
<tr>
<td>Gene for protein-53</td>
<td>$P^{53}$</td>
<td>17</td>
</tr>
</tbody>
</table>

**Evidence for tumor suppressor genes**

Evidence for tumor suppressor genes is varies and indirect. It includes the behavior of hybrid cells formed by fusing normal and cancerous cells, patterns of inheritance of certain familial cancers and ‘loss of heterozygosity’ for chromosomal markers in tumor cells.

**Retinoblastoma or RB$_1$ gene**

The RB$_1$ gene was the first tumor suppressor gene to be isolated. It was shown to be the cause of the childhood tumor of the eye, retinoblastoma. Mutations in the RB$_1$ gene have also been detected in breast, colon and lung cancers.

Similarly RB$_1$ gene encodes a protein designated as $p105$ (Molecular weight: 105,000). This protein also is found to suppress cell proliferation, and to prevent the activity of various oncogenes.

The oncogenes also provide an explanation for the multifactor origin of cancer. Thus viruses, chemical carcinogens, chromosome translocations, gamma-
rays, spontaneous mutations, and all such other factors may converge into one biochemical abnormality, the activation of oncogenes, which is later manifested as the malignancy.

Retinoblastoma takes two forms: familial (40% of cases), which exhibits the inheritance pattern for a recessive gene and which frequently involves both eyes; and sporadic, which does not run in families and usually only occurs in one eye. It was suggested a single gene. In the familial form of the disease, one mutated allele is inherited in the germ line. On its own this is harmless, but the occurrence of a mutation in the remaining normal allele, in a retinoblast cell, causes a tumor. Since there are $10^{-7}$ retinoblasts per eye, all at risk, the chances of a tumor must be relatively high. In the sporadic, noninherited form of the disease, both inactivating mutations have to occur in the same cell, so the likelihood is very much less and only one eye is usually affected. It should be noted that whilst familial retinoblastoma constitutes the minority of cases, it is responsible for the majority of tumors.

The two-hit hypothesis for retinoblastoma was also supported by evidence for loss of heterozygosis. The retinoblastoma gene (RB1) was provisionally located on human chromosome 13, by analysis of the genetics of families with the familial disease. By using hybridization probes for sequences closely linked to RB1, it was possible to show that the retinoblastoma cells of patients who were heterozygous for the linked sequences had only a single copy of the sequence that is there had been a deletion in the region of the supposed RB1 gene in tumor cells, but not in non tumor cells.

The RB1 gene was then isolated by determining the DNA sequence of the region of chromosome 13 defined by the most tightly linked marker sequences (specific chromosomal DNA sequences that are most frequently inherited with RB1). RB1 codes for a 110-kDa phosphoprotein that binds to DNA, and has been shown to inhibit the transcription of proto-oncogenes such as myc and fos. RB1 mRNA was found to be absent or abnormal in retinoblastoma cells. The role of RB1 in retinoblastoma was established definitively when it was shown that retinoblastoma cells growing in culture reverted to a nontumorigenic state when they were transfected with a cloned, normal RB1 gene. Unexpectedly,
RB1 mutations have also been detected in breast, colon and lung tumors.

**p53 gene**

P53 is the tumor suppressor gene that is mutated in the largest number of different types of tumor. When it was first identified, it appeared to have characteristics of both oncogenes and tumor suppressor genes. It is now known to be a tumor suppressor gene that may act in a dominant-negative manner to interfere with the function of a remaining, normal allele.

A part of short arm of chromosome 17 was shown to be deleted in various human cancers. This region is now known to contain an oncosuppressor gene, called P53. It is so called because the gene, encodes a phosphoprotein with molecular weight 53,000; with 375 amino acids in length. It can complex with other transforming proteins generated by other oncogenic viruses. (E.g. T antigen of SV 40, E6 of HPV-16) Most tumours have a complete absence of P53, whereas others show mutant nonfunctional P53. Normal P53 can suppress transformation ability of oncogenic viruses in vitro. It is also seen that P53 activates the expression of genes that suppress cell proliferation.

Confusingly, P53 has some of the properties of both oncogenes and of tumor suppressor gene.

- Many mutations (point mutations, deletions, insertions) have been shown to occur in the P53 gene, and all cause it to become oncogenic. Mutant forms of P53, when co-transfected with the ras oncogene, will transform normal rat fibroblasts. In cancer cells, P53 has an extended half-life (4-8 hrs) resulting in elevated levels of the protein. All this seems to suggest that P53 is an oncogene.

- A consistent deletion of the short arm of chromosome 17 has been seen in many tumors. In brain, breast, lung and colon tumors, where a P53 gene was deleted, the remaining allele was mutated. This suggests that P53 is a tumor suppressor gene.

The explanation seems to be that P53 acts as a dimmer. When a mutant (inactive) P53 protein is present it dimerizes with the wild-type protein to create an inactive complex. This is known as a dominant-negative effect. However, inactivation of the normal P53 gene by the mutant gene would not be expected to be 100%, since some normal dimmers would still form. Loss of the remaining
normal P53 gene may, therefore, result in a more complete escape from the tumor suppressor effects of this gene. (Lawrence A. Kaplan et.al.2003), (P.C.Turner et.al.1997).

1.1. D. Metallothionein as Tumor Marker

Metallothioneins are low-molecular weight intracellular proteins involved in many physiological processes, including zinc homeostasis. Among their many putative functions metallothionein may play a role in the intracellular storage of zinc. In addition, metallothionein can donate zinc-to-zinc finger proteins involved in cellular signaling and transcriptional regulation.

The metallothionein gene provides an example of how a single gene may be regulated by many different circuits. The metallothionein protein protects the cell against excess concentrations of heavy metals, by binding the metal and removing it from the cell. The gene is expressed at a basal level but is induced to greater levels of expression by heavy metal ions (such as cadmium) or by glucocorticoids. The control region combines several different kinds of regulatory element, and suggests the principle that when a promoter is regulated in more than one way, each regulatory event depends on binding of its own protein to a particular sequence.

In vivo studies have shown that metallothionein can supply zinc to the zinc finger transcription factors SPI, TFIIIA (which is required for RNA polymerase III to transcribe 5S rRNA genes), estrogen receptor and tramtrack. This reaction may occur by direct donation of zinc from metallothionein through a protein–protein interaction. Because of its high thiol content, metallothionein can also function as an antioxidant.

In vitro studies suggest direct reaction of hydrogen peroxide with the sulfhydryl groups of metallothionein. It has been shown that metallothionein, especially bound to zinc, has a higher capacity to protect against radiation – induced DNA damage than glutathione, and another antioxidant.

High levels of metallothionein bound to zinc and copper have been detected in liver during fetal development and the neonatal period. When there is a high requirement for zinc is cellular growth, a transient nuclear localization of
metallothionein has been demonstrated in human, rat and mouse hepatocytes during the fetal / neonatal period.

Studies have shown that metallothionein is induced during liver regeneration, a time of extensive hepatocellular proliferation and high requirement for essential metals such as zinc. This demonstrated the induction and nuclear localization of metallothionein in rat hepatocytes after partial hepatectomy. The induction of metallothionein is regenerating rat liver after hepatic injury with administration of carbon tetrachloride.

Ivor E. Dreosti says that Metallothionein are proteins consisting of a single polypeptide chain of 61-62 amino acids containing 20 cystein residues which contain several bivalent cations (zinc, copper, cadmium and mercury) bound through metal – thiolate linkages. Physiologically induction of MT appears to be principally involved in detoxification of heavy metals and metabolism of several essential trace elements. However, induction of MT by other stimuli including X, and UV irradiation and several anticancer therapeutic agents suggest it may also function to scavenge O$_2$ and OH radicals.

Accumulating evidence indicates that cells with low levels of intracellular MT are more susceptible to DNA damage and apoptotic death after exposure to stress stimuli including oxidative stress, whereas prior induction of MT appears to confer protection. Also relevant are the findings at changes in unicellular localization of MT from cytoplasm to the nucleus during early differential of myoblasts coincide with increased apoptosis newly formed myotubes (Benjamin Lewin et.al 1997).

**Classes of Biochemicals used as Tumor Markers**

This section is a review of several biochemical tests that have been used either as primary tumor markers or as secondary test to note invasion or dissemination of cancer. The types of analytes are listed in the box at the right and are discussed in terms of their clinical usefulness and applications. The Food and Drug Administration (FDA) regulates which tumor markers can be used. The current FDA-approved list of protein tumor markers includes CEA, AFP, PSA, PAP, CA-125, CA-15-3 and CA-27-29; the only tumor marker currently approved
for screening of the general population is PSA. As large clinical trials are completed, other such markers will be approved. Reviews that cover some of these assays in greater depth are recommended for further reading. (Vasudevan D.M et.al.19980).

Table 1.5: Classes of Biochemicals used as Tumor Markers

<table>
<thead>
<tr>
<th>Class of Biochemical</th>
<th>Examples</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased production of endogenous biochemicals</td>
<td>Hormones, enzymes, polyamines, and so on.</td>
<td>Confirmation, diagnosis, monitoring</td>
</tr>
<tr>
<td>Synthesis of biochemicals of previously quiescent genes</td>
<td>Oncofetal proteins, cell surface antigens, enzymes</td>
<td>Monitoring, prognosis</td>
</tr>
<tr>
<td>Receptors</td>
<td>Estriol receptor (breast cancer), androgen receptor (prostate cancer)</td>
<td>Prognosis, treatment</td>
</tr>
<tr>
<td>Modification of usual cell or organ function</td>
<td>Gamma-glutamyl transferase (GGT) or 5’-nucleotidase</td>
<td>Diagnosis</td>
</tr>
</tbody>
</table>

1.2. NUTRIENTS INVOLVED WITH CANCER PREVENTION

Undeniably, nutrition and cancer research will move from an observational to a molecular approach as knowledge about genomics and new technologies surfaces. Nutritional genomics offers opportunities to credential nutrients, that is, to determine which specific nutrient – related genetic and epigenetic changes bring about phenotypic changes that influence cancer risk. This knowledge should lead to the identification of molecular targets that can be manipulated for cancer prevention. In addition to describing interactions between selected nutrients and molecular targets, this review includes a discussion of certain nutrient-related genetic polymorphisms, that is, different allelic forms of genes that may influence cancer risk, the implications of nutritional genomics for food production, and future research direction. (John A. Milner et al., 2001).

Table 1.6: Partial List Of Nutrients That May Modify Cancer Risk
<table>
<thead>
<tr>
<th>Group</th>
<th>Nutrient</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vitamins</td>
<td>Vitamin D</td>
<td>Dairy product</td>
</tr>
<tr>
<td></td>
<td>Folic Acid</td>
<td>Vegetables</td>
</tr>
<tr>
<td></td>
<td>Vitamin A</td>
<td>Vegetables</td>
</tr>
<tr>
<td></td>
<td>Vitamin E(α-tocopherol)</td>
<td>Vegetables, Oils</td>
</tr>
<tr>
<td></td>
<td>Ascorbic Acid</td>
<td>Vegetables, Fruits</td>
</tr>
<tr>
<td>2. Minerals</td>
<td>Calcium</td>
<td>Dairy Product, Vegetables</td>
</tr>
<tr>
<td></td>
<td>Selenium</td>
<td>Vegetables, Fruits, Cereal, Grains, Meat, Fish</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>Red Meat</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>Vegetables</td>
</tr>
<tr>
<td>3. Carotenoids</td>
<td>Lycopene</td>
<td>Tomatoes</td>
</tr>
<tr>
<td></td>
<td>Lutein</td>
<td>Dark green vegetables</td>
</tr>
<tr>
<td></td>
<td>α – Carotene</td>
<td>Orange-yellow vegetables</td>
</tr>
<tr>
<td></td>
<td>β – Carotene</td>
<td>Orange-yellow vegetables</td>
</tr>
<tr>
<td>4. Flavonoids</td>
<td>Genistein</td>
<td>Soybean, Soy products</td>
</tr>
<tr>
<td></td>
<td>Resveratrol</td>
<td>Grapes, Red wine</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>Vegetables, Fruits</td>
</tr>
<tr>
<td></td>
<td>Rutin</td>
<td>Vegetables, Fruits</td>
</tr>
<tr>
<td></td>
<td>Tangeretin</td>
<td>Citrus fruits</td>
</tr>
<tr>
<td></td>
<td>Nobiletin</td>
<td>Citrus fruits</td>
</tr>
<tr>
<td></td>
<td>Catechins</td>
<td>Grapes</td>
</tr>
<tr>
<td></td>
<td>Epigallocatechin-3-gallate</td>
<td>Green tea</td>
</tr>
<tr>
<td></td>
<td>Anthocyanins</td>
<td>Vegetables, Fruits, Black tea</td>
</tr>
<tr>
<td>5. Organosulfur Compounds</td>
<td>Diallyl sulfide</td>
<td>Allium vegetables (Ex. Garlic Onion)</td>
</tr>
<tr>
<td></td>
<td>Allyl mercaptan</td>
<td>Allium vegetables (Ex. Garlic, Onion)</td>
</tr>
<tr>
<td></td>
<td>Allyl methyltrisulfide</td>
<td>Allium vegetables (Ex. Garlic, Onion)</td>
</tr>
<tr>
<td></td>
<td>S-Allylcysteine</td>
<td>Allium vegetables (Ex. Garlic Onion)</td>
</tr>
<tr>
<td>6. Isothiocyanates</td>
<td>Allyl isothiocyanate</td>
<td>Cabbage</td>
</tr>
<tr>
<td></td>
<td>2-Phenylethyl isothiocyanate</td>
<td>Cabbage</td>
</tr>
<tr>
<td></td>
<td>Benzyl isothiocyanate</td>
<td>Cabbage, Garden Cress</td>
</tr>
<tr>
<td></td>
<td>3-Methylsulfinyl propyl</td>
<td>Broccoli</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broccoli</td>
</tr>
</tbody>
</table>
### Figure 1: Specific Nutrients and Their Molecular Targets

This section provides clues to possible molecular targets for a few essential and nonessential nutrients. The diversity of molecular targets that are influenced demonstrates the complexity and breath of nutrient interactions and supports the concept that nutrients act within numerous biochemical and molecular cascades, thereby serving as significant modulators of cancer risk.

Figure one reveals that the pleiotropic effects of nutrients cannot be attributed to a single regulatory mechanism but are likely a manifestation of nuclear and cytoplasmic events that regulate the abundance and/or activity of specific proteins. Fluctuations in these proteins can lead to changes in overall cellular metabolism and can markedly influence the proportion of cells that are dividing, undergoing apoptosis, or differentiating. (John A. Milner et al., 2001).

---

**Nutrients**
- Vitamins
- Minerals
- Carbohydrates
- Fat
- Protein
- Flavonoids
- Organosulfur Compounds

**Table:**

<table>
<thead>
<tr>
<th>7. Indoles</th>
<th>Indole -3- carbinol</th>
<th>Cruciferous Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indole -3- acetonitrile</td>
<td>Cruciferous Vegetables</td>
</tr>
<tr>
<td></td>
<td>D-Carvone</td>
<td>Citrus Fruits, Oils</td>
</tr>
<tr>
<td>9. Phenolic Acids</td>
<td>Curcumin</td>
<td>Turmeric, Curry, Mustard</td>
</tr>
<tr>
<td></td>
<td>Caffeic acid</td>
<td>Fruits, coffee Beans, Soybean</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid</td>
<td>Fruits, Soybeans</td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid</td>
<td>Fruits, Coffee, Beans, Soybeans</td>
</tr>
<tr>
<td>10. Chlorophylls</td>
<td>Chlorophyll</td>
<td>Green Vegetables</td>
</tr>
<tr>
<td></td>
<td>Chlorophyllin</td>
<td>Green Vegetables</td>
</tr>
</tbody>
</table>
The focus of this work is limited to selenium and vitamin D because they represent different classes of nutrients and potential molecular targets related to cancer risk have been identified. Vitamin D and selenium are being investigated in chemoprevention trials. Population-based chemoprevention strategies will benefit from observations linking nutrients with specific cancer related molecular targets.

1.2. A. VITAMIN D₃

Vitamin D is ambiguous because, in the presence of sunlight, skin cells are capable of synthesizing a sufficient supply of the vitamin from a derivative of cholesterol. Since a dietary source is not required in this case, the vitamin is more correctly classified as a pro hormone (i.e., a precursor of an active hormone). The pro hormone form of vitamin D, whether synthesized in the body or obtained from the diet, is converted to the active form by enzymes in the liver and kidneys. The active form then is delivered to target organs.

The amount of sun exposure individuals need to produce vitamin D depends on skin color, age, time of day, season of the year, and geographic location. Experts recommend that people expose their hands, face, and arms two to three times a week to 30 to 50% of the amount of sun needed to cause sunburn.
In other words, for a person who would sunburn is just a half-hour, 10 to 15 minutes of exposure is recommended. This sun exposure is effective for vitamin D synthesis only if done between about 8 a.m and 4 p.m in all areas of the country. It is not effective at all in the winter in northern climates. Some people may be able to use the vitamin D that was stored from summer months in their fat cells, but most people in northern climates should find a alternative vitamin D sources in the winter months.

**Vitamin D formation in the skin**

Synthesis of vitamin D begins with pro vitamin D (7-dehydrocholesterol), located in the skin. During exposure to sunlight, one ring on the molecule breaks open creating pre vitamin D₃. Over the next few hours, pre vitamin D₃ undergoes a transformation aided by body heat to form vitamin D₃, this change allows vitamin D₃ to enter the bloodstream; bound to a protein it is now on its way to becoming a hormone. The more pigment in the skin, the less vitamin D₃ is made. Melanin acts as a natural sunscreen.

**Absorption and Formation of Vitamin D from Food**

Following the consumption of vitamin D-containing foods, about 80% of vitamin D is incorporated into micelles in the small intestine and then absorbed and transported to the liver by chylomicrons through the lymphatic system. Patients with chronic fat-malabsorption syndromes (e.g. cystic fibrosis, Crohn’s disease, and celiac disease) have trouble absorbing vitamin D and may develop a deficiency.

**Metabolism, Transport, and storage of Vitamin D**

When vitamin D (either synthesized in the skin or consumed from food or supplements) enters general circulation, it is bound to a protein. The formation of the hormone form of vitamin D from its precursor occurs in the liver and kidneys. In the liver, the vitamin is hydroxylated on carbon 25, converting it to 25-OH-D. This inactive form circulates in the blood for weeks. The next stop is the kidney, the principle site for the production 1, 25(OH)₂ D, also known as calcitriol or the
hormone form of the vitamin, and is active for about 1 day. Patients with chronic kidney failure have very low concentrations of circulating 1,25(OH)\(_2\) D. They are routinely treated with 1, 25(OH)\(_2\) D. Thus, the kidney is the organ in which activation of vitamin D occurs.

Once vitamin D enters general circulation, it can be stored in fat tissues for later use or converted to 25-OH-D in the liver. When there is a shortage of calcium in the blood, the parathyroid glands increase production of parathyroid hormone (PTH). Parathyroid hormone then increases the production of 1, 25(OH)\(_2\) D in the kidney. Eventual excretion of vitamin D takes place mostly via the bile, with small amounts leaving via the urine.

**Dietary sources of vitamin D**

Because some people may not receive enough sun exposure to generate sufficient active vitamin D for the body’s needs, they need to pay attention to dietary sources. Actually, few foods contain appreciable amounts of vitamin D.

The densest sources of vitamin D (μg/kcal) are fatty fish (e.g., sardines and salmon), fortified milk, and some fortified breakfast cereals. In the United States, milk is fortified with 10μg (400IU) per quart. Although eggs, butter, liver, and a few brands of margarine contain some vitamin D, large servings must be eaten to obtain an appreciable amount of the vitamin; thus, these foods are not considered significant sources.

Foods of animal origin contain vitamin D\(_3\), whereas plants contain a slightly different pro vitamin D called ergosterol, or vitamin D\(_2\). Metabolism of vitamin D\(_2\) in the body yields 1, 25-dihydroxy ergocalciferol. This compound has vitamin D activity in humans. The usual form of vitamin D in supplements is D\(_2\).

**Vitamin D needs**

The food and nutrition board has set an adequate intake for vitamin D. The adequate intake for vitamin D is 5μg/day (200 IU/day) for people under age 51 and increases to 10μg/day (400 IU/day) for people between 51 and 70 and
15μg/day (600 IU/day) for older Americans. Young, light-skinned people can produce enough vitamin D from casual sun exposure on just the face and hands. The marker used to determine the adequate intake for young adults is the concentration of 25-OH-D in the blood, the precursor to the active form of the vitamin. For older persons indices of bone maintenance are also used.

Infants are born with a sufficient supply of vitamin D to last about 9 months. At or before that time, a breastfed infant should be regularly exposed to some sunlight or treated with a supplement under a physician’s guidance. Pasteurized milk is usually fortified with vitamin D as are infant formulas.

**Vitamin D Toxicity**

Toxicity occurs from excess supplementation, not from sun exposure or milk consumption. Anyone contemplating or using supplements of vitamin D should consider a dosage no higher than 10 to 20 μg/day or (400 to 800 IU/day). The upper level set for vitamin D is 50μg /day (2,000 IU/day), based on development of an effects similar to those of too little vitamin D by causing too much calcium to move from the bones to the blood and then to the urine for excretion. This excess can also be toxic to the liver. Other symptoms of hypercalcemia also occur. Severe vitamin D toxicity in infants causes mental retardation, narrowing of pulmonary arteries and the aorta, and changes in facial characteristics. Calcium deposits in organs cause local cell death. Additional effects then result as a large number of cells die. Vitamin D can be a very toxic substance if consumed at amounts above 250μg/day (10,000 IU / day). That is over 16 to 50 times the adequate intake set for adults of various ages. It is especially toxic to infants.

Vitamin D is only for people who fail to produce enough from exposure to sunlight. Most people can synthesize adequate vitamin D by the action of sunlight on the skin. Older people and breastfed infants are at risk of a vitamin D deficiency. Vitamin D₃ is later activated by the liver and kidneys to form the active hormone 1, 25(OH)₂ D. This hormone increases calcium absorption in the intestine and works with other hormones to maintain proper blood calcium concentrations and calcium metabolism in bones and other organs in the body.
The hormone 1, 25 (OH)₂ D is also an important factor of cell differentiation in many tissues of the body. Fish oils and fortified milk are significant food sources of vitamin D. An excess of vitamin D is quite toxic, especially, during infancy; continuous intakes greater than 50μg/day should be consumed only with a physician’s guidance. Sun exposure poses no risk of vitamin D toxicity.

**Essential Role of Vitamin D₃ in Cancer**

Vitamin D and its metabolites appear to act through a variety of molecular targets to inhibit carcinogenesis is the consequence of vitamin D binding to epithelial growth factor with subsequent inhibition growth and increased differentiation in normal and malignant cells. Studies with breast cancer cell lines (i.e., murine CS-2 and human MCF-7) also indicated that depressed proliferation and enhanced apoptosis contribute to the preventive activities associated with vitamin D.

Because vitamin D acts as a hormone, interest in its ability to bind with estrogen receptors (ERs) in breast and prostate tumors has surfaced. Nolan and colleagues reported that apoptotic events caused by vitamin D occur in ER (+) or ER (-) breast cancer cells, suggesting that the effect is independent of estrogen status. Vitamin D also interacts with androgens via androgen receptors (AR) through the action of the VDRs insulin and insulin like growth factors IGF-I are recognized to be involved in promoting growth and development of breast tumors. Vitamin D reverses the mitogenic effects of insulin and IGF-I by interruption of late-growth signaling pathways, rather than by a direct effect or by binding to insulin or IGF-I receptors.

The ability of vitamin D to inhibit growth and induce apoptosis in tumor cell lines independent of P⁵³ tumor suppressor gene signaling pathways is of particular interest. In an investigation of apoptotic pathways in MFC-7 and T47D cells, it was determined that vitamin D activates apoptosis through a Bcl-2 regulated pathway, independent of known cascades of P⁵³ status. Another molecular target for vitamin D is the c-myc proto-oncogene, which induces proliferation and tumor growth.

Overall, considerable evidence reveals a direct functional consequence of
vitamin D and its metabolites on a number of physiological processes. Receptor polymorphism is clearly an important determinant in the cellular response to 1, 25 (OH) _2_ D. Such evidence provides insights into how receptor genotype-phenotype association can account for a plethora of cellular responses. Variances in responsiveness among individuals may occur on the basis of naturally occurring variants of a single gene. For a full understanding of the response to 1, 25 (OH) _2_ D, a more detailed understanding of its interactions with cofactors is essential. It is certainly conceivable that these cofactors will vary among responses and cell types.

**Functions of Vitamin D**

The principal function of vitamin D is to maintain serum calcium and phosphorus concentration within the range that supports neuromuscular function, bone calcification, and other cellular processes. By helping maintain the blood calcium concentration within the normal range, 1, 25(OH) _2_ D plays another vital role: maintaining the function of neuromuscular junction. The function of 1, 25(OH) _2_ D is fundamentally to control the production and action of several calcium-binding proteins in the small intestine. One protein in particular is activated by 1, 25(OH) _2_ D and is responsible for the rate of flow of calcium across the intestinal mucosa. Another function of 1, 25(OH) _2_ D is to increase the absorption of phosphorus.

The 1, 25(OH) _2_ D reacts with a specific receptor in target tissue. (This receptor belongs to the family of putative steroid hormone zinc-finger receptors). The receptor binds with 1, 25(OH) _2_ D to a vitamin D receptor, the retinoic acid receptor & RXR (described earlier) to form complex. This then interacts with specific DNA sequences known as vitamin D–responsive elements, 1, 25(OH) _2_ D complex either enhances or inhibits the transcription of vitamin D-responsive genes. If the hormone-specific gene is turned on, it can produce the related mRNA transcripts, which are then translated into several different proteins, including the calcium-binding protein.

Following is a summary of vitamin D’s biological functions

- Vitamin D maintains serum calcium levels by mobilizing calcium and
phosphorus from bone stores during a time of calcium shortage.

- 1, 25(OH)₂ D induces stem cell monocytes to become mature osteoclasts. Osteoclasts are bone-degrading cells, which release calcium into the blood.
- Vitamin D is not needed for bone calcification but is responsible for maintaining extracellular calcium and phosphorus concentrations in a supersaturated state. This results in the mineralization of bone. The cells that actually form bone are called osteoblasts.
- Human epidermal cells have nuclear receptors for 1, 25(OH)₂ D, so it effects the proliferation and differentiation of skin cells. At present, there are 20 different cell types in the human body that are sensitive to 1, 25(OH)₂ D.
- Vitamin D is also capable of influencing differentiation in some cancer cells, such as skin, bone and breast cancer cells. Indeed, adequate vitamin D status has been linked to a reduced risk of developing breast, colon and prostate cancer. Studies are not yet conclusive, though and are mainly supported by in vitro experiments.

1.2. B. SELENIUM

Selenium first attracted the attention of scientists in the 1930s, when it was found to cause a chronic poisoning of livestock. This resulted from the animals’ consuming plants that were grown on high-selenium soils. The significance of selenium in human nutrition became evident in the late 1980s, when Chinese scientists reported that selenium supplementation prevents the development of Keshan diseases, which causes a form of heart disease. The known biological roles of selenium are diverse. It is vital normal development, growth and metabolism.

Absorption, Transport, Storage and Excretion of Selenium

Selenium enters the body in many ionic forms. Most selenium in foods is bound to derivatives of the amino acids methionine and cysteine. Therefore, the two major forms of selenium that enter the body are as selenomethionine, derived ultimately from plants, and selenocysteine from animals. Because these substances are readily absorbed, the bioavailability of selenium is considerably higher than that of iron and zinc. About 50 to 100% of dietary selenium intake is absorbed, and it is not affected by selenium nutritional status. Since no physiological mechanism appears to control selenium absorption, selenium has a
definite potential for toxicity.

Not much is known about the transport of selenium. What is known is that selenium is made available for use when the particular amino acid it is bound to is catabolized. The selenium can then be incorporated into macromolecules, transported to various organs, or excreted. Homeostasis of selenium in the body is achieved through excretion, mainly via the urine and faces. Studies show that the urinary excretion of selenium increases as dietary intake increases. Storage of selenium primarily is found bound to the amino acid methionine and as part of glutathione peroxide enzyme. Both are found throughout the body.

**Selenium in Foods**

Fish, meat (Especially organ meat), eggs, milk and shellfish are food animal sources of selenium. Grains and sources of nuts and seeds grown in soils containing selenium are good plant sources.

Foods providing the highest nutrient density for selenium, ($\mu g$/kcal) are tuna, whole-wheat bread, ham, eggs, oatmeal, white bread and related flour-based products, beef and chicken.

**Selenium Needs**

The estimated average requirement for selenium is 45 $\mu g$/day for men and women ages 19 to 70 years. This is based on the amount of selenium needed to maximize glutathione peroxidase activity. The estimated average requirement is increased by 20% to account for individual variability to yield a RDA for both men and women ages 19 to 70 years of 55 $\mu g$/day.

**Toxicity of Selenium**

Excess selenium can be toxic. The upper level is 400 $\mu g$/day for adults 19 years and older, based on overt signs of selenium toxicity, such as hair loss and high blood concentrations. Daily intakes as low as 1 to 3 mg can cause toxicity symptoms if taken for many months. These signs and symptoms besides hair loss include a garlicky odour of the breath, nausea, diarrhea, fatigue and changes in fingernails, toenails, rashes and cirrhosis of the liver may also develop.
Essential Role of Selenium

Although investigation of selenium’s role in health promotion has focused on its antioxidant activity, it has diverse biological functions, including the ability to suppress cell proliferation, enhance immune response, alter the metabolism of carcinogens, and induce apoptosis. Providing selenium in its inorganic (e.g., selenite and selenate) or organic (e.g., Selenocysteine and selenomethionine (Se Met) forms has been found to meet nutritional needs.

Research on the gamut of molecular targets for selenium has supported its ability to function as an antioxidant and alter several events that lead to changes in cell proliferation, differentiation, and apoptosis. Considerable attention has been devoted to its role in the thioredoxin system, a major antioxidant system. The activity of thioredoxin reductase (TR), a seleno enzyme, has also been linked to nuclear transcription factor nuclear factor-kB (NF-kB) activation through its ability to regulate thioredoxin concentrations. NF-kB is an inducible oncogenic nuclear transcription factor that responds to the redox state of the cells and has a vital role in inducing genes involved in a number of physiological processes, including those associated with cytokines, growth factors, cell adhesion molecules and immunoreceptors.

TR specifically reduces oxidized thioredoxin to its reduced form using NADPH. Selenium availability is a key factor that determines overall TR activity in cells in culture and in vivo, providing supplemental selenium to HT-29 human colon cancer cells grown in serum-free medium markedly increased TR activity. Recent studies by Ganther and lp provide evidence that supra physiological exposure to selenium does not affect the activity of this enzyme. Thus, although it may be important to aberration in neoplastic cells, it may not be a target for exaggerated selenium intakes.

Role of Selenium in cancer

Various forms of selenium markedly retard the growth of neoplasms. This may relate to a direct genetic effect of selenium, such as inhibition of DNA synthesis and induced DNA strand breakage. For instance, selenite has been shown to increase DNA strand breakage, increase cdc2 / cdk2 kinase
activities without changing cyclins bound to cdk2, and arrest cell growth in the S/G\textsubscript{2}/M phase. The seleni-compound methyleneselenocysteine also has been reported to increase the number of double-strand DNA breaks, thus inducing apoptosis and arresting cell growth in the G\textsubscript{1} phase.

In addition, recent studies reveal that selenium availability can alter DNA methylation. Davis and colleagues found that DNA isolated from Caco-2 cells, a human colon cancer cell line, not treated with selenite was significantly hypomethylated compared with that from cells treated with 1 or 2 \(\mu\)M selenite. In addition, methylation of the p53 promoter region of Caco-2 cells decreased when cells were cultured in the absence of selenite. In an \textit{in vivo} study, rats fed selenium-deficient diets had significantly hypomethylated liver and colon DNA compared with rats fed diets containing selenium at 0.1 or 2.0 \(\mu\)g as selenite or selenomethionine. Thus alterations in DNA methylation may be potential mechanisms by which selenium may alter tumorigenesis. Although these findings are exciting, additional studies are needed to determine whether DNA from other tissues is also influenced and how other dietary components might affect this response.

Se-Met is the predominant form of selenium in dietary supplements and is the form most widely used in prevention trials. Because methionine-t RNA cannot distinguish methionine from Se-Met, incorporation of Se-Met into tissue proteins may account for its lower toxicity than other forms of selenium. The efficacy of Se-Met has also been found to depend on the intake of methionine, again pointing occur among the various dietary components. Nevertheless, even when methionine intake is adequate, Se-Met can influence pathways related to carcinogenesis, such as interruption of polyamine biosynthesis, which is required for normal cellular proliferation and development.

Although interactions among nutrients have been inadequately examined, a few examples of negative and positive interactions with the response to selenium are available. Vitamin C has been reported to reduce selenium’s effectiveness against chemically induced colon cancer. The significance of such interactions may be even more pronounced, because selenium has been shown to enhance the ability to inhibit chemically induced mammary cancer in experimental animals.
Introduction

Greater attention to all components of the diet and elaboration of their interactions should make possible specific and appropriate recommendations for the general population and allow for recommendations tailored to specific subgroups or individuals. The importance of such understanding is exemplified in the recent review by Alaejos and colleagues in which they presented data indicating that several factors, including α-tocopherol, β-carotene, retinol, and vitamin C might account for variability in the response to dietary selenium.

Functions of Selenium

Currently, the best understood role for selenium is as a cofactor for a major form of the enzyme glutathione peroxidase. Selenium also plays a role in thyroid hormone metabolism and likely has other metabolic functions, which have yet to be clearly established.

Glutathione peroxidase participates in a process that metabolizes peroxides into less toxic alcohol derivatives and water. That peroxides tend to become free radicals, which in turn can attack and break down cell membranes, causing cell damage. As a cofactor for glutathione peroxidase, selenium is important for protecting heart cells and other cells against oxidative damage. Selenium also may aid immune function via activity of glutathione peroxidase.

Recall that vitamin E also functions to prevent attacks on cell membranes by free radicals. Thus, vitamin E and selenium work together. Selenium participates in an enzyme system that prevents free radical production by reducing peroxide concentration in the cell, and vitamin E can stop the action of free radicals once they are produced. Thus, an adequate selenium intake spares some of the body’s need for vitamin E, as it reduces the peroxide load in a cell. The electron-seeking compounds, especially free radicals, can alter DNA. Alterations in DNA are known to cause cancer. Because of selenium’s ability to reduce free radical production, adequate intake of this mineral may be important in preventing cancer. In fact, recently, people with a history of skin cancer (basal cell or squamous cell carcinomas) were treated with an oral supplement of 200 µg of selenium / day or a placebo, there was no effect on the further development of cancer of the skin; however, there was a significant reduction in development of
other cancers, such as lung, colon, rectal, and prostate. This study has peaked interest in selenium in cancer prevention and also has prompted follow-up studies. Another study using selenium (and vitamin E) supplements in men who have an-enlarged prostate gland is in the planning stages. The dose of selenium will be 200 $\mu$g / day and 400 IU / day for vitamin E. Vitamin E is part of the protocol, since some supplement trials have hinted at a protective effect against prostate cancer.

1.3. Objectives of the Present Study

The present study is an attempt to understand the role of antioxidant selenium and dietary micronutrient vitamin D$_3$ when given together could be effective in vivo as chemopreventive agents against experimentally induced rat liver carcinogenesis. A brief outline of the essential objectives taken into consideration includes.

- To see dietary essential micronutrients like selenium in combination with vitamin D$_3$’s desirable effect on the carcinogen induced hepatic dysphasia.
- Secondly to extend the study of role of selenium and vitamin D$_3$ on the antioxidant status and on the xenobiotic metabolizing enzyme pattern in the liver as well as effort was made to ascertain whether they could exert their chemopreventive action on pathophysiological aspect during neoplastic transformation.
- Finally, the anticarcinogenic potential of these two dietary micronutrients has been assessed by their ability to modulate certain molecular (genotoxic) marker in order to have a meaningful understanding of the cellular and molecular bases of selenium and vitamin D$_3$ actions on anticarcinogenicity and genomic stability during the early stages of chemically induced hepatocarcinogenesis in rats.
The observations embedded in this thesis mainly focuses a defined role of selenium and vitamin D$_3$ in combination for inhibiting the carcinogenic regimen induced by a potent hepatocarcinogen like diethylnitrosamine (DEN) in the rat liver. The study is a clear indication of the role of combined supplementation of selenium and vitamin D$_3$ in metallothionein gene expression, cellular proliferation and the antioxidant status during preneoplasia to combat the process of carcinogenesis and may rank an unique attempt for therapeutics in near future.