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Secondary Malignancies Induced by Radiation Exposure: Clinical Examples and Current Experimental Findings

By Roy Vongtama and David A. Corral, MD

Radiation therapy is a proven, effective modality for the treatment of many forms of cancer. Since its introduction as part of the standard of oncologic care, radiation has also been questionably associated with the evolution of secondary malignancies. Secondary malignancies are a serious and potentially lethal concern not only for the patient, but also for the physician, who must take this issue into account when deciding whether to recommend radiation as a part of therapy. The process by which radiation induces neoplastic transformation is not well understood. However, significant advances have been made in recent years in the basic research of radiation-induced malignant transformations which have indicated possible underlying mechanisms. This article will briefly explore the incidence of secondary malignancies in several organ systems and examine the current experimental theories of the mechanism of radiation-induced malignancy.

History

Radiation has been suggested as a potential cause of nearly every type of malignancy. With this being said, however, two facts must be recognized that significantly modify this statement. The first is that the levels of radiation exposure that have been shown to be clearly associated with secondary malignancies range from one to 10 Gray (Gy), exposure that is much lower than dosages typically used for therapy. Within this range of radiation dosages, a linear dose response between exposure and cancer incidence can be seen. Studies have failed to show the same linear dose response with the higher (therapeutic) levels of radiation, although increased risk has been demonstrated.[2] It has been postulated that at therapeutic dosages, the high level of cell death may result in the destruction of potentially malignant cells which would otherwise have been transformed by the radiation. The second fact which must be recognized is that certain malignancies seem to be more radiosensitive than others. This article

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will focus on thyroid cancer, breast cancer, and soft tissue sarcomas—three malignancies which are highly radiosensitive.

Thyroid Carcinoma

Thyroid carcinoma is a classic example of a malignancy which has been shown in numerous studies to be induced by radiation exposure. In a study by Schneider and colleagues, 5.9% of patients who had received radiation therapy for benign head and neck conditions developed a secondary thyroid cancer 3-42 years after radiation therapy, with a median time to diagnosis of 10 years following radiation. Interestingly, the clinical course of the radiation-associated thyroid carcinomas did not vary from thyroid cancer diagnosed in patients with no prior radiation exposure. In children, the risk of radiation induced thyroid cancer inversely correlated with their age at the time of exposure. Those who were exposed to radiation before age 5 had a five-fold higher incidence of secondary thyroid cancer than those exposed after 10 years of age.[4] As seen with adults, the clinical course did not vary significantly from that of non-radiation induced thyroid carcinomas, with the majority of diagnoses peaking 15-20 years after radiation.[5]

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Breast Cancer

Radiation has also been suspected as a cause of breast cancer. Similar to thyroid carcinoma, the risk appears to be inversely proportional to age, with children exposed to radiation at a young age having a higher risk later in life. Within the low-dose range, it has also been shown that there is a linear relationship between dose and relative risk. Again, the clinical course of these radiation associated breast cancers seems to be no different than that seen in breast cancer patients who had not been previously exposed to radiation.[6]

Soft Tissue and Bone Sarcomas

Sarcomas of soft tissue and bone associated with radiation exposure are well reported in the literature.[7,8] For example, Souba and colleagues reported 16 cases of sarcoma arising in previously irradiated sites located in the chest wall. Because these patients received radiation therapy for non-sarcomatous tumors, these data support the theory that radiation can play a significant role in the oncogenic process of secondary malignancies. In general, as with non-radiation associated sarcomas, radiation associated sarcomas have a poor prognosis due to their normally advanced stage upon discovery, with the only effective therapy being surgical removal, and average five-year survival reaching only 30%.[7]

Experimental Model for the Study of Radiation-Induced Tumors

As can be seen from the above examples, a case can be made that radiation is a potent inducer of cancer, especially at low levels. However, this has yet to be proven convincingly in preclinical studies. Much of the current knowledge behind radiation-induced malignant transformation has been discovered in the last 10 years. The model discussed in this article was developed by Mendonca and associates and utilized a hybrid line of cells consisting of a tumorigenic cell line (HeLa) combined with human skin fibroblasts. This model is an excellent experimental model for the study of radiation-induced tumors for two reasons. As discussed above, many radiation associated malignancies are sarcomas—tumors which result from malignant transformation of mesenchymal cells such as fibroblasts. Also, the model follows Knudson's "two hit" hypothesis of carcinogenesis that was initially described in the retinoblastoma model. Briefly, Knudson's hypothesis states that a cell with a genetic locus (encoding a tumor suppressor) which contains a mutated allele may be phenotypically normal as long as a nonmutated allele is normally expressed. For malignant transformation to occur, the second allele

must be altered. This results in a loss of heterozygosity and allows the preexisting genetic mutation to lead to malignant transformation.

The HeLa line used for the hybrid cell line has been shown to be inherently tumorigenic and produces the antigen intestinal alkaline phosphatase (IAP), which can be used as a marker to signal tumorigenic activity. The human skin fibroblast line has the characteristics of being non-tumorigenic and having tumor suppressing activity. Combined together, the hybrid line, HeLa* human skin fibroblast, is an actively tumor suppressing cell line that contains on average four alleles per chromosome, two from the non-tumorigenic fibroblast and two from the tumorigenic HeLa line. The production of IAP, the HeLa neoplastic marker, is actively suppressed by gene products of the normal human skin fibroblast. This system provides a very precise means to detect neoplastic transformation in the hybrid cell line (i.e., if the fibroblast's tumor suppressor alleles are knocked out, the production of IAP will resume and can be quantitated).[9,10] *(Note: the actual hybrid cell line used for the experiment, CGL1, is a descendant of the original hybrid HeLa* fibroblast line.)*

In the Mendonca study, hybrid cell lines were irradiated with 7 Gy of gamma rays at 2.1 Gy/min and then trypsinized, counted, and seeded into flasks containing standard growth medium. These cells were then fed, plated, and left to grow for 7, 9, 11, 13, 15, 17, 19, and 21 days, at which time they were fixed and stained for IAP with Western Blue reagent. If a cell had been transformed by the radiation exposure, it would begin to produce IAP. Neoplastic frequencies for each day (7 through 21) were measured by counting the number of neoplastic foci per total number of surviving cells; A higher frequency translated into a higher rate of malignant transformation. Interestingly, IAP positive foci did not begin to appear at a significant frequency until the 11th day. The IAP positive finding demonstrated that malignant transformation did occur, but occurred in a delayed fashion on day 11. This coincided with the reduced plating efficiency of cells seen, which also began on the 11th day. Plating efficiency refers to the ability of transferred cells to grow in appropriate media. With regard to plating efficiency, non-irradiated controls plated out at a relatively constant rate of 60-80%. The irradiated samples initially start at 11% on day 4, recover to 35-45% on day 9, and steadily decline thereafter. The reduced plating efficiency of irradiated cells demonstrated that a significant proportion of cells were unable to successfully replicate, possibly implying that they had undergone significant genetic damage that manifested itself on day 11.

The temporal coincidence of IAP appearance (i.e., malignant transformation) and reduced plating efficiency (i.e., genetic damage) on day 11 was a significant discovery that led to additional investigations into the mechanism of each. It was discovered that the tumor suppressor loci that were lost with radiation exposure were found to be on fibroblast chromosomes 11 and 14.[9] These two loci were found to be necessary but not sufficient for neoplastic transformation, as control lines which had lost either of these suppressor loci did not develop neoplastic characteristics (namely, IAP production). Mendonca et al proposed that the delayed loss of tumor suppressor function was not a discrete event, but rather the buildup of heritable damage over the course of replicative cycles in which the final result was loss of tumor suppressor function.

Recent Laboratory Discoveries

In the most recent study published in *Cancer Research,* Mendonca and coworkers investigated the significant reduction in plating efficiency seen in the irradiated cell population.[10] It was demonstrated that these cells undergo a delayed apoptosis which cause the drastic reduction in plating efficiency. Apoptosis is a genetically mediated form of cell death induced by various stimuli. Radiation is known to be a potent epigenetic apoptotic stimulus.[11] The upregulation of apoptosis around day 11 was demonstrated by several methods previously shown to be indicative of apoptosis, some of which will be listed here. DAPI staining was done, which indicated abnormal morphology consistent with apoptosis. Endonuclease mediated DNA strand breaks consistent with apoptosis were also seen by TUNEL assays. Western blot showed increased levels of p53 and Bax, proteins which have been shown to be proapoptotic. In short, all techniques used were in agreement, thereby lending credence to the theory that plating efficiency was decreased because of induction of apoptosis. Mendonca et al proposed that the actual trigger for apoptosis is the same for loss of tumor suppressor function: it is not a single event, but rather the buildup of heritable damage over the course of replicative cycles.[11] Termed genetic instability, the damage becomes great enough to trigger apoptosis.

Summary

With all this being said, the question remains: how does radiation induce neoplasia in these hybrid cells? A summary of the work of Mendonca et al gives a possible answer. Radiation is given, producing immediate cell death and also sublethal genetic damage. The remaining HeLa* fibroblast cells begin to replicate. The significant changes begin approximately on day

10; at this time, the hybrid cells have undergone approximately 12 population doublings. A buildup of genetic mutations occurs over these replicative cycles. This genetic instability can have two outcomes, one of which is the induction of apoptosis (and therefore cell death) that occurs for the majority of the cells. The second outcome is that a small subset of these irradiated cells lose their tumor suppressor genes (11 and 14) but either evade apoptosis or have not had enough accumulated damage to trigger apoptosis. Thus they become neoplastic.

Patients who have been recommended to receive radiation as a primary form of treatment are often intrinsically afraid or at least aware of the potential for long-range side effects. This hesitancy is well founded: secondary cancers associated with radiation exposure are body wide and have a poor prognosis, as shown above. Clearly the work done by Mendonca et al provides significant advances in the quest to understand radiation-induced cancers. The future lies in further experimentation to better understand the mechanisms involved using more complex biological models. The ultimate goal is to understand the complete process of radiation-induced malignant transformation which will translate into clinical knowledge and help physicians determine the most effective therapeutic strategies which confer the least possibility for secondary malignancies. *(Roy Vongtama is a Medical Student, School of Medicine and Biomedical Sciences, University at Buffalo.)* ❖

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Stealth Liposomes: Delivering Chemotherapy Undercover

By Mohanakrishnan Menon, MD

Optimizing the therapeutic benefits of chemotherapeutic agents while reducing the potential toxicity has led to novel drug delivery systems. A promising advance is the use of liposomes, which selectively deliver a greater dose of chemotherapy to tumor tissue.

Anthracyclines are active against a variety of tumors but are associated with significant cardiac toxicity in the form of congestive heart failure in approximately 5% of patients. Encapsulating the free drug, doxorubicin in stealth liposomes, which evade detection by the reticuloendothelial system (RES), is an attempt to reduce cardiac toxicity and increase tumor response to the drug.

Liposomes are the small vesicles of spherical shape with an average diameter of 50-100 nm that are produced from neutral phospholipids and cholesterol. They can carry small drug molecules, proteins, nucleotides, and even plasmids. The lipid bilayer entraps an aqueous core that can carry hydrophilic drugs while the hydrophobic core of the phospholipid bilayer can incorporate lipid soluble drugs.[1]

Up to 70% of a dose of conventional liposomal preparations is taken up by the RES in the liver, spleen, and marrow, limiting its usefulness in targeting non-RES tissue. Stealth liposomes are so named because they evade detection and uptake by the RES, thus substantially increasing their half-life.

Stealth liposomes, as shown in the Figure, have their surface coated with a hydrophilic polymer, such as polyethylene glycol, by a process often known as pegylation. The polymer is thought to attract a water shell, resulting in a decrease in the absorption of protein opsonins on the

liposomal surface. This, in turn, decreases the liposome uptake by the reticuloendothelial system, and extends the circulation time.

Passive Targeting

Lengthening the circulation time of liposomes appears to increase their localization into tissue capillaries by increasing permeability in a variety of solid tumors. Liposomes are usually smaller than 100 nm in diameter and are able to pass through these pores. This is supported by colloid gold-containing stealth liposomes which can be seen microscopically in the tumor tissue. Normal tissue with intact capillaries is essentially impermeable to the liposomes. This method of increasing the drug concentration in tumor tissue is known as passive targeting. The volume of distribution is much smaller for liposomes as compared to free drug because the liposomes are confined to the vasculature or central compartment and tissues with increased vascular permeability.

The pharmacokinetic properties of doxorubicin 25 mg/m^2 administered as free drug, in conventional liposomes and pegylated liposomes, to patients with a variety of solid tumors is given in the Table.

Mechanism of Action

On localization of the liposome to the tumor tissue, there is a slow, sustained release of the drug in its free form, which appears to be responsible for the therapeutic effects. There is no evidence to suggest uptake of liposomes by tumor cells. In patients with AIDS-related Kaposi's sarcoma (AIDS-KS), the doxorubicin level was 5-11 times higher with pegylated liposomal doxorubicin (PLD) in lesions biopsied 72 hours after administration of equivalent doses of free doxorubicin and non-pegylated liposomal drug.[5]

Table

Pharmacokinetic Properties of Doxorubicin 25 mg/m^2

Formulation	1st t1/2 (h)	2nd t1/2 (h)
Free doxorubicin[2]	0.07	8.7
Conventional liposomal doxorubicin[3]	0.29	6.7
Pegylated liposomal doxorubicin[2]	3.2	4.5

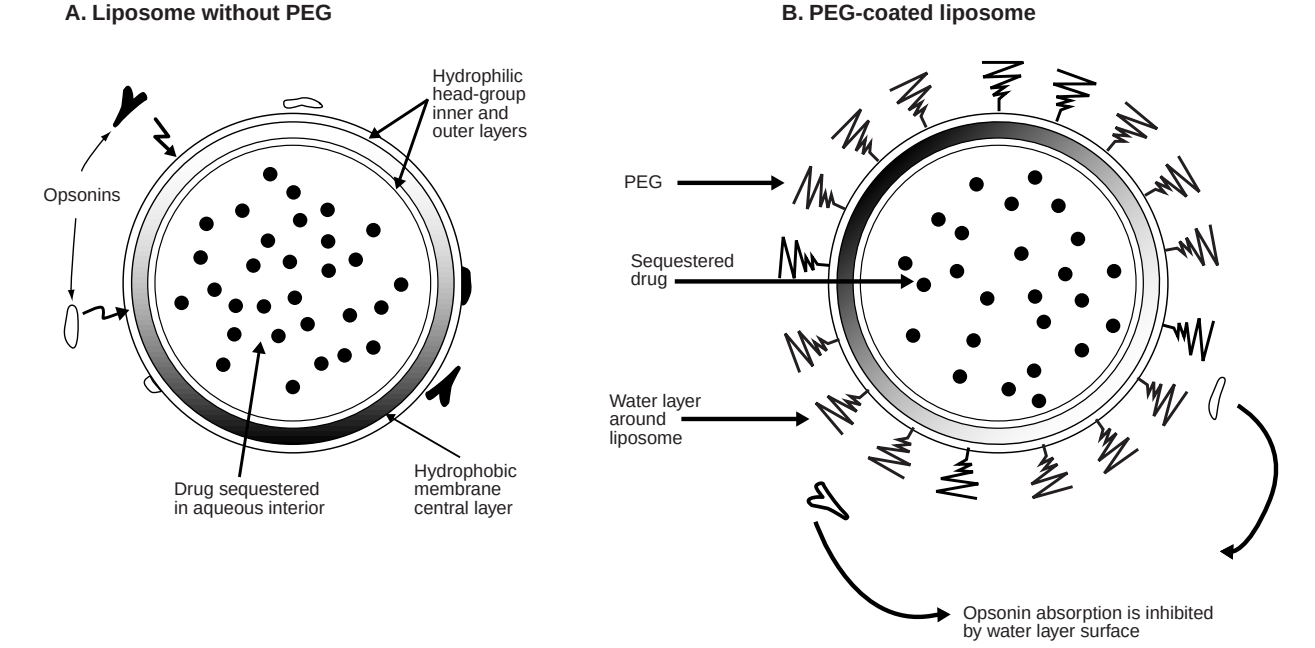
Clinical Applications

PLD (Doxil or Caelyx) is approved by the FDA for treatment for Kaposi's sarcoma and refractory ovarian carcinoma. Its role is being explored in Phase II trials in a variety of cancers including breast, lung, and soft tissue sarcoma.

Available Treatment Data

Kaposi's Sarcoma: PLD is FDA approved for first line treatment of Kaposi's sarcoma. A phase III Trial with 241 patients compared PLD 20 mg/m^2 to bleomycin 15 units/m^2 and vincristine 2 mg every

Figure



three weeks for six cycles showing a higher response rate of 58% for the PLD arm vs. 23.3% for the other arm.[4] There was a lower incidence of nausea, vomiting, and peripheral neuropathy in the PLD arm. The incidence of mucositis, however, was higher in the PLD arm. In another trial comparing PLD 20 mg/m^2 to doxorubicin 20 mg/m^2, vincristine 1 mg and bleomycin 20 mg/m^2 every 14 days for six cycles showed a response rate of 45.9 for the PLD arm vs. 24.8 for the combined arm.[5] The toxicity profile also favored the PLD arm.

Ovarian Cancer: PLD is FDA approved for ovarian cancer refractory to front-line regimens using paclitaxel and platinum agents that is either progressing on therapy or within six months of completion of previous therapy. In a Phase II trial including 35 patients with refractory ovarian cancer, an overall response rate of 26% was observed to PLD 50 mg/m^2 every three weeks. Responses lasted for 8-21 months and compared favorably to other salvage regimens, including carboplatin (4-7 months) and paclitaxel 5-6 months.[6]

Breast Cancer: Phase II trials are currently ongoing for metastatic breast cancer. A phase III trial reported at the 35th Annual Society Meeting of the American Society of Clinical Oncology (ASCO) this year by Batist and colleagues used the non-pegylated liposomal form of doxorubicin (TLC 99) compared to doxorubicin in combination with cyclophospamide in 297 patients with metastatic breast cancer. It showed no significant difference in response rates, median progression-free survival, and duration of survival. However, a lower incidence of congestive heart failure (0 vs 5 patients) and myelosuppression was noted. The regimen used 600 mg/m^2 of cyclophospamide and 60 mg/m^2 of both doxorubicin dosage forms every three weeks.[7]

A Phase II trial to assess safety and tolerability with paclitaxel by P. J. Woll used a regimen of paclitaxel 175 mg/m^2 every three weeks with Caelyx 30 mg/m^2 every three weeks or 60 mg every six weeks. Toxicities were hand foot syndrome, mucositis, and neutropenia.[8]

Lung Cancer: Koletsky and associates reported at the Society Meeting of the ASCO this year on 28 patients who received PLD as second-line treatment of advanced non-small-cell lung carcinoma after platinum-based therapy. No objective responses were seen, but disease stabilization for more than six months was seen in three patients.[9]

Soft Tissue Sarcoma: A randomized Phase II trial by EORTC presented at the Society Meeting of the ASCO reported the results from a study of 94 patients treated either with doxorubicin or Caelyx. The latter, as per preliminary results, was shown to have equivalent activity with a better toxicity profile in the form of lesser incidence of alopecia and Grade 4 neutropenia. Cardiac toxicity resulted in stopping treatment in one patient on doxorubicin.[10]

Side Effects and Toxicity Profile

Cardiac Toxicity: Doxorubicin cardiomyopathy occurs in about 5% patients. The incidence increases sharply beyond a lifetime cumulative dose of 450-550 mg/m^2. Clinical experience with PLD beyond cumulative doses of 500 mg/m^2 is limited. The manufacturer does not recommend using a dose greater than 550 mg/m^2. Currently available patient data and animal models suggest a reduction in cardiotoxic potential using PLD. Theoretical reasons for possible reduced toxicity include reduced uptake in the cardiac tissue and lower peak levels of doxorubicin due to a slow release of liposomal contents.

Myelosuppression: Leucopenia was the dose-limiting adverse effect in patients with AIDS-KS occurring in up to 60% of the patients. Concomitant factors in this population include HIV disease and numerous medications.

Hand Foot Syndrome or Palmar Plantar Erythrodysesthesia (PPE): An increase in the incidence of PPE has been seen with PLD. It is characterized by skin eruptions on the palms and soles, with pain, inflammation, and in some patients, ulceration, and desquamation. The precise mechanism of developing PPE is unclear. It may be related to prolonged exposure to extravasated liposomes due to breakage of small capillaries in the pressure sensitive areas of the palms and soles. Alternatively it may be caused by tissue accumulation with a prolonged exposure to keratinocytes. The likelihood of developing PPE can be reduced either by spacing the dosage intervals or reducing the dose.

Incidence of alopecia was reduced, as well as the vesicant reaction to local infiltration in comparison to free doxorubicin.

Other Pegylated Products

Due to the prolonged circulatory half life, pegylated liposomes have been evaluated in other situations as well. Pegylated forms of cisplatin SPl-077 are reportedly effective in murine models and are still in the process of early clinical evaluation. Pegylated forms of isoniazid (INH) and rifampin are being evaluated for treatment of tuberculosis. Similar forms of amphotericin are being evaluated in fungal infections. Radiolabelled technitium and indium in pegylated liposomes are being explored for anatomic localization of inflammation.

Conclusion

The pegylated liposomal form of doxorubicin has a proven benefit and is approved for therapy of Kaposi's sarcoma and refractory ovarian carcinoma. It's role in other tumors and other clinical situations is still being evaluated. Other agents encapsulated in stealth liposomes will hopefully find a variety of applications in the future. *(Dr. Menon is a fellow in Medical Oncology at Roswell Park Cancer Institute, Buffalo, NY.)* ❖

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Cyclooxygenase-2 and Colorectal Cancer

By Robert D. Lewis and David A. Corral, MD

In the early 1900s, it was discovered that two isoforms of the enzyme cyclooxygenase (COX) take part in the biologic conversion of arachidonic acid to prostaglandin. This important discovery eventually led to further studies that demonstrated a clear physiologic difference between COX-1 and COX-2, with different implications concerning the effects of COX-1 and COX-2 in different organs, tissues, and disease states.[1]

Research into COX-1 and COX-2 expression was performed using monoclonal antibodies and messenger RNA hybridization. It was discovered that COX-1 is constitutively expressed in all tissues producing prostaglandins which regulate several key physiologic functions. In stark contrast, COX-2 expression is almost undetectable in normal cells. However, during inflammation, COX-2 expression is increased in inflammatory cells (such as macrophages, etc.).[3] These important results would seem to support the theory that COX-1 performs homeostatic functions in tissues, whereas COX-2 is generally an inducible enzyme that is responsive to inflammatory stimuli. The upshot of this theory is that it has lead to investigation to determine whether specific inhibition of COX-2 would result in the blockade of pain and inflammation without affecting the homeostatic function of COX-1.[3] Additionally, this selective blockade of COX-2 would avoid the deleterious side effects associated with COX-1 inhibition produced by nonsteroidal anti-inflammatory drugs (NSAIDs), namely gastrointestinal (GI) bleeding.

Results of clinical and pharmacologic studies support the evidence that COX-2 inhibition is sufficient to replicate the analgesic and anti-inflammatory effects seen with NSAIDs while limiting the GI side effects. Additionally, the recent success and popularity of the new COX-2 inhibitor drugs such as celecoxib (Celebrex) would seem to support this view as they have

clear indications for treatment of rheumatoid and osteoarthritis.

COX-2's complex array of physiologic roles has been recently discovered. It has been shown to play a role in prostaglandin production in the brain and kidney. There has also been recent speculation that it might have a direct role in Alzheimer's disease as well as colorectal cancer.

COX-2 has a clear role in kidney function. Experiments by Harris in rats have shown that the kidney always exhibits low but measurable concentrations of COX-2 messenger RNA.[1] Harris' studies of the localized COX-2 expression to regions of the kidney mainly involved in sodium reabsorption and renin release which ultimately results in the release of aldosterone, thus promoting sodium reabsorption in the kidney. Additionally, COX-2 expression was observed in the macula densa and the loop of Henle. It must be understood that these observations made by Harris are animal experiments and have not been replicated in humans.

Laboratory Data

Animal experiments in rats have shown COX-2 to be constitutively expressed in the brain of developing rats. COX-2 is detectable in the hippocampus by postnatal day 5 and in the cortex by day 21.[1] In adult rats, COX-2 expression has been shown to be regulated by physiologic synaptic activity. It has been shown in animals that a multitude of stimuli can upregulate COX-2 in the brain. It has been theorized in the past that Alzheimer's disease involves stress to the brain, followed by activation of microglia that express COX-2 in a cerebral inflammatory process. This cascade, it has been hypothesized, ultimately leads to cell death and loss of memory.[1] Of additional interest is the fact that NSAIDs have been shown in studies to retard the progress of Alzheimer's disease. A study by Rich et al showed that patients with Alzheimer's disease who took NSAIDs daily exhibited a slower disease progression than non-users.[1] Thus, it can by hypothesized that the NSAID inhibition of COX-2 production might be slowing the disease progression leading to these positive results.

Over the past 10 years, significant evidence has been accruing that shows a link between cyclooxygenase-2 and colorectal cancer/adenomas in humans. In 1994, Eberhart and colleagues conducted experiments measuring COX-1 and COX-2 messenger RNA levels isolated from human colorectal cancers and adenomas which they compared to normal mucosa. These results showed increased levels of COX-2 messenger RNA levels in 12 of 14 carcinomas, compared with normal mucosa.[4] Additionally, this experiment also showed that COX-2 gene expression is low to undetectable in normal colorectal mucosa. Finally, in their study of colorectal adenomas, the precursor lesion to carcinoma, it was seen that many adenomas showed upregulation of COX-2 messenger RNA. It is imperative to note that the study showed equal expression of COX-1 in both normal and neoplastic colorectal tissue derived from humans.[4]

Other experiments by Fujita at the University at Tokyo took these previous findings a step further and sought to determine if there was a link between size/invasion of colorectal tumors in relation to levels of COX-2. The results of their experiment demonstrated that COX-2 levels are significantly higher in tumors with larger diameters and greater surface areas. Additionally, the COX-2 indices were also higher in tumors with deeper invasion.[5] The experiment was not able to show any correlation between COX-2 levels and the presence of metastatic disease. These results would seem to imply that COX-2 levels increase during the progression of colorectal adenomas to carcinomas as most colorectal carcinomas are derived from smaller adenomas which progress in size, volume, and diameter.

An experiment in 1997 by Sheng et al set out to test the hypothesis that COX-2 is involved directly in intestinal tumor development. Sheng and colleagues were responding to studies which reported that daily NSAID intake decreased the risk of colorectal cancer by 40-50%. It was well known at this time that NSAIDs inhibited COX-1 and COX-2, but they wanted to directly link COX-2 inhibition with suppression of colorectal cancer.

Sheng et al addressed this issue by implanting human colorectal cancer cells (HCA-7) that constitutively expressed COX-2 in mice and measured the response to treatment with a highly selective COX-2 inhibitor SC-58125.[2] Sheng et al also used a colon cancer cell line that lacked COX-2 expression to test drug selectivity. The results showed that treatment with the COX-2 inhibitor suppressed tumor growth by 85-90% in the cell line with high COX-2 expression, but had no effect on the cell line that lacked COX-2 expression.[2] Thus, the results suggested that COX-2 inhibitor drugs, which are currently on the market for the treatment of inflammatory conditions, may in the future allow for progress in the treatment and even prevention of colorectal cancer.

Summary

The results of recent research concerning COX-2 shows that there is a direct link between COX-2 and colorectal cancer. The articles reviewed above would seem to support the theory that there is a direct link between COX-2 levels and the size, invasion, and growth of colorectal cancer. Additionally, the results of this research would seem to support the theory that the new COX-2 inhibitor drugs, such as celecoxib and rofecoxib (Vioxx), might have utility in the treatment and prevention of col-

orectal cancer as they work directly on an important factor potentially involved in progression and development, without causing the unwanted GI side effects seen with NSAIDs.

Recently, on Aug. 30, it was announced by the FDA that celecoxib a COX-2 inhibitor, will get priority review for the prevention of colorectal adenomatous polyps in patients with familial adenomatous polyposis (FAP). FAP is an uncommon hereditary condition which can cause hundreds of thousands of adenomatous polyps to develop in the rectum during adolescence and early childhood. Left untreated, virtually all patients will develop colon cancer by age 40-50. No drugs are currently approved for use in FAP. Hopefully, if the results are positive, testing of these drugs may be extended to prevention and treatment of non-hereditary colorectal cancer afflicting millions of Americans each year. The reviewed studies would seem to indicate a strong possibility that COX-2 inhibitors could be helpful not only in FAP but also in slowing and suppressing the growth of malignant carcinomas. *(Robert D. Lewis is a medical student at the State University of New York at Buffalo School of Medicine.)* ❖

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Phospholipase A2, Eicosanoids, and Apoptosis

By David P. Schuster, and Thomas Nicotera, PhD

Products formed during the metabolism of arachidonic acid (AA) play a deterministic role in regulating cell growth, function, and survival. In order for proper cellular functioning to occur, release of arachidonate from membrane phospholipids, as well as the constellation of enzymes directed toward eicosanoid metabolism must remain intact. Phospholipids acted upon by phospholipase A2 (PLA2) release free fatty acid from the sn-2 position. The sn-2 position is frequently occupied by unsaturated fatty acids, such as AA. Arachidonate can be processed by cyclooxygenase pathways (COX) or lipoxygenase pathways (LOX) to produce prostaglandins and leukotrienes, respectively. Arachidonate metabolites are potent mediators of a broad range of physiologic phenomena.

Perturbations in eicosanoid metabolism have been observed in a number of tumors and tumor-derived cell lines, and an increased ability to metabolize AA may contribute to the aggressiveness of tumor cells. Several enzymes have been identified that can significantly affect the concentrations of eicosanoids in cells. The mitogen inducible form of cyclooxygenase (COX-2) is elevated in the highly invasive MDA-MB-231 breast cancer cell line, as compared to the less invasive MCF-7 cell line.[1] Specific inhibition of COX-2 can result in a decreased tumor growth rate and an increase in tumor cell apoptosis.[2,3] Furthermore, the expression of COX-2 is elevated in gastric carcinomas and in neoplastic colon tissue as compared to adjacent, noncancerous tissue. Elevated COX activity should result in the generation of prostaglandins, and prostaglandins (particularly prostaglandin E) have been implicated as mediators in angiogenesis.[3] Exogenous AA has been demonstrated to be mitogenic in human prostatic cancer cells,[4] and there is evidence that leukotrienes can prevent programmed cell death in prostate tumor cell lines.[4,13] These data suggest that altered regulation of arachidonate metabolism can provide multiple mitogenic stimuli.

Metabolism of AA in prostate tumors has been of interest in part because of epidemiological studies suggesting a role for dietary fats in the etiology of prostate cancer. Arachidonate is less abundant in the membrane of malignant prostate cells as compared to benign tissue.[12] Addition of exogenous AA to prostate tumor cells results in an increase in production of leukotrienes[4,5] and prostaglandins.[15] Also, malignant tissue samples obtained during radical prostatectomy were shown to have elevated activity of PLA2 when compared to benign prostate tissue.[12] No evidence of de novo synthesis of unsaturated fatty acids in prostate was found,[12] and enzymes dedicated to the allocation of unsaturated fatty acids, AA-CoA hydrolase and lysophospholipid acyl transferase, were unchanged in malignant tissue as compared to benign tissue. This suggests that malignant cells have constitutively high PLA2 activity, and strip AA from membranes in malignant tissue.

Ability of Prostate Tumors to Establish Metastases

The availability of substrate for cyclooxygenase and lipoxygenase may be a critical factor in the ability of prostate tumors to establish metastases. In our lab, inhibition of PLA2 resulted in apoptosis in two well characterized prostate tumor cell lines, LNCaP, and PC3 cells. The more aggressive PC3 cells had a higher PLA2 activity and were more sensitive to inhibitor-induced apoptosis than the LNCaP cells. Exogenous AA generates an abundance of 5-HETE in these cell lines.[4,5] LNCaP and PC3 cells are constitutive producers of the leukotriene precursor 5-HETE,[5] and leukotrienes are suggested to impair programmed cell death in these cells.[6] Other investigators report that blocking the lipoxygenase (LOX) pathway induced rapid apoptosis in both cell lines.[5,6] It was also observed that apoptosis induced in human protaste cells by LOX inhibition was reversed by the addition of 5-HETE.[6] This would suggest that AA liberated from membranes and metabolized by the lipoxygenase pathway has a cytoprotective effect in these tumor cell lines. In this case, constitutive elevation of PLA2 activity creates a proliferative advantage. Some sublines of these tumor cells selected for metastatic potential,[7] also have high PLA2 activities, as determined in our laboratory. We are currently exploring the sensitivity of these sublines to inhibitor-induced apoptosis.

Treatment of Eicosanoid Metabolism

Three major enzymes have been considered so far in this treatment of eicosanoid metabolism, PLA2, COX, and LOX. As is the case with COX-1 and COX-2, LOX and PLA2 have a variety of isoforms. LOX isoforms differ in their specificity for particular ethylene moieties in the eicosanoid backbone, and characteristics attributed to 5-HETE have also been attributed to products of 15-LOX.[13] Similarly, PLA2 activity is a composite of a variety of isoforms with different substrate specificity. The cPLA2s have an affinity for phospholipids with AA in the sn-2 position and require calcium for the translocation of active enzyme to the membrane. The sPLA2s require calcium to facilitate vessicular release, but do not have a preference for AA containing phospholipids. Furthermore, cPLA2 has been reported to be constitutively activated in PC3 cells,[14] which may account for the relatively high PLA2 activity in these cells.

Conclusion

Recently, messenger RNA from a new class of PLA2s, the calcium-independent iPLA2, has been sequenced.[8-10] Several splice variants of this species of RNA have been identified, and at least two of the splice variants code for truncated proteins with no consensus sequence for PLA2 activity.[8] Active forms of this enzyme show specificity for phospholipids with AA in the sn-2 position, and one active form requires ATP.[10] Truncated, inactive proteins predicted to exist are proposed to play a regulatory role in a multimeric iPLA2 complex. It has been documented that cells originating from different tissues maintain different ratios of these splice variants.[9,10] We have also obtained preliminary data that suggest aberrant expression of this iPLA2 occurs in prostate tumor cells. Regulation-deficient iPLA2 isoforms in prostate tumors could result in the over production of cytoprotective eicosanoids, angiogenic catalysts, or mitogenic stimulation. Detailed information about AA metabolism in these cells may reveal markers of tumorigenicity, or new therapeutic approaches to this disease. *(David P. Schuster is a graduate student in Molecular and Cellular Biophysics at Roswell Park Cancer Institute, Buffalo, NY; Dr. Nicotera is a Cancer Research Scientist in Molecular and Cellular Biophysics at Roswell Park Cancer Institute, Buffalo, NY.)* ❖

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Funding News

What Are Contract Research Organizations and Why Are They on My TV?

You may have noticed their advertisements on the evening news, or heard them on the radio on the way to or from work. They are Contract Research Organizations (CROs) and they have found a way to tap into the money provided by pharmaceutical and medical technology corporations eager to complete clinical trials to move their products to the medical marketplace. What once was the realm of the academic health center is rapidly becoming the domain of small and large businesses that are creating their own specialty niche by running clinical trials. With the rapid proliferation of new medications and surgical and medical devices comes a need for proof of safety and efficacy in the clinical arena. In order for the pharmaceutical and biomedical technology companies to recover their investments in developing these products and begin to generate income, they need to obtain FDA approval by the most rapid and efficient means possible. These corporations are recognizing more and more that academic health centers are likely to take longer to complete this task than their profit-motivated private-sector counterparts. As a result, the CROs have been successfully pulling trials, patients, and dollars away from academic centers.

In his article entitled "Academic Centers, CROs Vie For Industry Dollars" in *The Scientist*, Paul Smaglik points out that in an environment of diminishing revenues due in large part to HMOs, some academic centers have begun to fight back by forming their own mini-CROs. Smaglik cites Duke University's initiatives taken with pharmaceutical and device manufacturers which have focused on the relatively specific area of testing cardiac-based products.[1] Smaglik goes on to point out that the CROs have responded in kind by developing specialized arms to address specific areas. As an example, the Quintiles Transnational Corp., a $1.2 billion company in 1998, generates revenue by conducting general medical trials, but also conducts trials in specialized areas such as oncology, pediatrics, and women's health.

Corporations such as Quintiles can use their familiarity with marketing strategies to their advantage, as evidenced by their collaboration with former Surgeon General C. Everett Koop, using his Web site to help enroll patients in their trials. Academic centers will, no doubt, need to improve their marketing skills to keep up with corporations who know their way around this environment very well.

Is this just a problem for the clinical academic researcher who wants to perform Phase II or III clinical trials? Not any more. Smaglik also points out that Covance, Inc., is expanding into preclinical trials in areas such as toxicology, which were previously the territory of government-sponsored research at academic institutions.

From the vantage point of academic medicine, the old adage "if you can't beat them, join them" has been transformed into "you better beat them in a proactive manner or join them early before they take away your trials." The medical research environment today requires not only clinical and scientific skills, but also a knowledge of marketing and economics. *(By David A. Corral, MD.)* ❖

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The Cancer Treatment Research Foundation

The Cancer Treatment Research Foundation (CTRF, Web site: www.ctrf.org) is a non-profit national medical research organization dedicated to expediting the translation of research findings directly into treatment options for cancer patients. CTRF's mission is to raise, manage, and distribute resources for innovative cancer research and educational programs. CTRF is the beneficiary of generous corporate underwriting, including Cancer Treatment Centers of America, which underwrites the entire administrative and most of the fund raising expenses. CTRF's primary consideration in granting support is the immediacy of improvement in treatment options and quality of life for cancer patients. CTRF seeks to fund progressive clinical approaches that may stand alone or complement conventional treatments. CTRF is not a traditional source of funds and believes that many other resources are available for incremental improvements in cancer therapy. For example, CTRF will not support extensions of conventional or traditional chemotherapy such as phase III/IV trials. Also, while transitional research of a pre-clinical nature which is within a year of clinical trials will be considered, basic research will not be supported.

The overall goal of the **Clinical Investigation Grant program** of CTRF is to fund young investigators who are without support from the NIH or other cancer research agencies working on innovative research relevant to cancer therapy (e.g., new agents, immunotherapy, biological response modifiers, gene therapy), clinical nutrition, quality of life, and cancer education. Established clinical researchers who wish to embark on innovative studies with novel, new, or pilot projects may also apply and should demonstrate that their project is a departure from ongoing, funded work. Awarded funds may be used to support the proportion of salary devoted to the project by the principal investigator and other key personnel. Funds may be used for supplies and non-reimbursable research-related patient care costs, including extraordinary laboratory and imaging studies. Equipment will be funded only in extremely rare circumstances. The amount of the award varies with the availability of funds. Previous awards have ranged between $10,000 and $125,000/year. The maximum period of funding is for two years.

A Preliminary Grant Application may be downloaded at the CTRF Web site or obtained by calling (847) 342-6484. ❖

CME Questions

22. Tumor suppressor loci lost in irradiated HeLA* fibroblast hybrids were localized to fibroblast chromosomes:

a. 9 and 12.
b. 10 and 13.
c. 11 and 14.
d. 12 and X.

23. In order to escape the reticuloendothelial system, "stealth liposomes" are coated with:

a. a hydrophobic polymer such as chondroitin sulfate.
b. a hydrophilic polymer, such as polyethylene glycol.
c. nonspecific polyclonal antibodies.
d. monoclonal antibodies.

24. Phospholipase A2:

a. converts eicosanoids to phospholipids.
b. synthesizes phospholipids from fatty acids.
c. converts saturated to unsaturated fatty acids.
d. liberates arachidonic acid from phospholipids in the cell membrane.

25. COX-2 expression:

a. is constitutively high in all tissues.
b. is increased by the inflammatory reaction.
c. is not affected by inflammation.
d. is induced by SC-58125.

In Future Issues: Genomic Instability