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14. ABSTRACT

There is significant evidence that indicates an increased risk for developing secondary lung cancer in women treated with radiotherapy for breast cancer and an additional risk for women who smoke. It is important to determine molecular markers for the susceptibility of breast cancer patients to lung cancer to improve information for treatment decisions. Markers in lung cancer may correlate with cancers that develop from radiotherapy alone, in combination with cigarette smoking, or with smoking alone. This project examines p53 mutations, p16 and Ecad methylation, and Estrogen Receptor Alpha as markers of susceptibility to lung cancer after breast cancer. Of 343 tumors assayed, 31 had p53 mutations, 23 had p16 or Ecad methylation, and 186 were Estrogen Receptor Alpha positive. Data generation has been completed for this study and work has progressed to statistical analysis. There may be problems with power due to small sample size in the analysis of subcategory interactions, however, primary hypotheses should be addressable. As this study continues, it will accumulate as many as 600 cases and the hypotheses will be more confidently assessed, providing information that may be useful in defining subpopulations sensitive to radiation therapy.

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Breast cancer, lung cancer, secondary cancer, epidemiology, gene-environment interactions

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Introduction

Radiotherapy has become a standard treatment for breast cancer however, few studies have examined individual susceptibilities to risks from radiation exposure. Lung cancer following breast cancer has been associated with radiation exposure and this increased lung cancer risk has been shown to be even higher with tobacco exposure. Identification of molecular markers of radiation exposure may allow the distinction of groups of women susceptible to secondary lung cancer or multiple cancers and of women more significantly affected by smoking. Additionally, identification of molecular markers in breast and lung tumor tissue may suggest a common etiology for breast cancer and secondary lung cancer. To study the risks associated with radiotherapy for breast cancer, we are using samples from the Swedish Cancer Registry, which contains information on approximately 95% of all cancer cases in Sweden.

We are looking at breast and lung tumor tissue for mutations in p53, which is involved in a radiation response pathway and is strongly associated with DNA damage from smoking. We are also comparing p53 mutations between women who did or did not receive radiotherapy and between smokers and nonsmokers with or without radiotherapy. Methylation of DNA is a key factor in the regulation of gene transcription and has been shown to contribute to carcinogenesis by blocking transcription of tumor suppressor genes. Based on this knowledge, we will be assessing the methylation of several genes known to be involved in cancer progression. Additionally, we are performing immunohistochemical assays to describe Estrogen Receptor Alpha (ERA) expression in breast and lung tumors. This project may have significant clinical impact by providing additional information on risk levels to women choosing a breast cancer therapy. Additionally, this research may provide new data on the susceptibilities of women with multiple primary cancers and on hormone related gender differences in cancer risk

Body

Background

Radiation exposure is indicated as a risk factor for several cancers, but the study of radiation risks has been limited because of difficulties in measuring individual exposures and subsequent susceptibilities. Occupational studies have demonstrated that uranium and plutonium workers have an increased risk of lung cancer (1-3) as well as Japanese atomic bomb survivors, but individual exposures are difficult to precisely quantify. Studies of secondary cancer risk after radiotherapy are better able to establish radiation doses and link them to risk. Women with a history of breast cancer treated with radiotherapy in these studies had a secondary lung cancer risk that increased 2-3 fold for nonsmokers and 30 fold in smokers who receive radiation therapy (4,5). Overall, radiotherapy causes about 7-9 additional cases of lung cancer per 1000 women over a 10-year period (4,6). While the data is consistent, studies have been small and lacking in reliable information on radiation and smoking dose (7) and none have explored the effects of radiation and smoking in women with breast cancer at a molecular level.

p53

The p53 gene is an appropriate subject for the study of cancer etiology, exposure, and susceptibility because of its many roles in cellular processes, including maintenance of genomic stability, apoptosis, DNA repair, and cell cycle control (8-12). p53 is upregulated in response to DNA damage by radiation (13-15), and cell lines with mutated p53 are hypersensitive to point mutations following radiation therapy (16). Examples of specific carcinogen exposures linked to cancers via p53 mutation

mechanisms include ultraviolet (UV) light exposure and skin cancer (17-19) and dietary aflatoxin B1 exposure and liver cancer (20,21). The p53 tumor suppressor gene is the most commonly mutated gene in cancer; it is mutated in 40% of breast tumors (22,23) and 50% of lung tumors (24,25). p53 mutation frequency varies by tumor site and histological type (14), indicating that cancers occur through different pathways and due to different exposures at the cellular level. In breast cancer, studies indicate that the p53 mutation spectrum differs by race and geography (26,27) suggesting differences in etiology that might be environmental or genetic (28-30). In lung cancer, the type and frequency of p53 mutations have been correlated with smoking, and a study of uranium workers found a p53 mutational spectra in the workers' lung tumors that differed from the lung tumors of smokers (31). Studies in other populations, however, have not found differences in mutational spectra (54-58). Although there is conflicting data, significant evidence suggests that there may be a difference in the mutational spectra of women who develop secondary lung cancer with smoking and radiation in contrast to smoking alone (31,59).

Estrogen Receptor

Steroid receptors are required for normal lung maturation and function. ERA has a well-known role in the progression, treatment, and prognosis of breast cancer and may play a role in lung cancer as well. Gender differences in risk of lung cancer suggest that hormones and their receptors may influence the biology of lung cancer. Previous studies of ERA expression and gender in lung cancer present varying reports, perhaps due to the lack of standardized techniques at the time (62-64, 69). A recent study found both ERA and Estrogen Receptor beta in normal and tumor tissue in lung, with lower levels of both in the tumor samples (68). Studies conflict on whether ERA has a prognostic value for lung carcinoma (51,60,61). However, there is significant evidence indicating an increased risk of adenocarcinoma of the lung with estrogen replacement therapy and an even higher risk in smokers who receive estrogen replacement therapy (53). These findings support the possibility that exogenous hormones play a role in the etiology of lung cancer in women. The significant implications of estrogen acting in the development of lung tumors and concerns about prior studies call for an examination of the presence of ERA in women with breast and secondary lung tumors. There may be a shared hormonal etiology for these breast and secondary lung tumors and a potentially greater role for ERA expression in the development of secondary lung cancer in nonsmoking, radiation treated women. Additionally, women with two primary cancers may represent a phenotype of increased estrogenicity or sensitivity.

Methylation

Methylation of DNA is an epigenetic feature of DNA known to contribute to regulation of gene expression and to maintaining genome stability. DNA methyltransferases add methyl groups to the 5' cytosine residues of the dinucleotide CpG (32-34). Abnormal methyl patterns are consistently found in cancer, including hypermethylation of promoter regions and genome-wide and gene-specific hypomethylation (35). Both DNA hypermethlation and hypomethylation occur early in tumorigenesis and are thought to contribute to tumor progression, but whether abnormal DNA methylation is a consequence or a cause of cancer has not been established (36).

Several genes involved in breast and lung cancer are known to have abnormal methylation patterns. The DNA repair enzyme *O^6-methylguanine-DNA methyltransferase* has been shown to be frequently inactivated in lung cancer by aberrant promoter methylation (42,53). The *BRCA1* gene is involved in maintenance of genomic integrity and studies have found it to be hypermethylated in 13%-29% of sporadic breast cancer (43,44). The tumor suppressor gene *p16* is involved in cell cycle control and hypermethylation of its promoter has been shown to decrease p16 expression (45). 20%-40% of

breast cancers have hypermethylated *p16* (46,47). The *estrogen receptor (ER)* is downregulated in some breast cancers and lack of ER is associated with a poor prognosis. Hypermethylation of the *ER* promoter region has been detected in 63% of ER negative breast tumors (48) and has also been reported in lung tumors (49). *E-cadherin* is a transmembrane glycoprotein this is involved in cell adhesion. Aberrant CpG island methylation of *E-cadherin* has been found in breast cancer at a rate of 26-48% (70-72); in lung cancer, the rate is 18-33% (67). The *retinoic acid receptor ß (RAR-ß)* is a ligand activated transcription factor that is known to exert antiproliferative, differentiating, and apoptosis-induced effects on different types of tumors (73). In primary breast cancer specimens, 21-58% of specimens are hypermethylated at *RAR-ß* (74-75) and in nonsmall cell lung cancer, 40% of specimens are hypermethylated *RAR-ß* (67). Specific methylation patterns have been demonstrated to be associated with lung cancer and breast cancer and can provide valuable information on pathways in the development and progression of tumors.

Research Accomplishments

Pathology Review

Pathology review was anticipated as part of the tissue processing for this project, but after initial review, it was determined that a more thorough review of cases in this study was necessary to establish the primary status of case lung tumors. All case lung tumors included in the study were recorded with the Swedish Cancer Registry as primary tumors. In an effort to insure validity of the study, a review of cases was conducted by a pathologist at GU. This review utilized H&E stains from the breast and lung tumors of each case and they were examined in pairs to compare tumor morphology. After this review, 51 cases were deemed questionable as primary tumors and required supplemental information and additional review. To conduct this review, the GU pathologist traveled to the KI to meet with the pathologist involved with the study in Stockholm. An additional review was conducted with a multi-head microscope to facilitate coordinated viewing and supplemental immunohistochemistry stains were reviewed when available, including ERA and Thyroid Transcription Factor 1 (TTF-1). Reports have demonstrated that TTF-1 is an excellent marker for lung cancer (76% staining of adenocarcinomas) and does not stain breast cancer (77-82). We are using the 8G7G3/1 clone (83), a monoclonal antibody from Zymed Laboratories (San Francisco, CA). The TTF1 antibody was optimized for antigen retrieval and dilution of the antibody at 1:300 for staining at room temperature for 1 hour. 11 tumors in this review had positive TTF-1 staining (additional TTF-1 staining was done on other cases, but the other lung tumors were considered primary based on morphology alone and did not require the additional review). Each case was discussed and a consensus was reached on assignment of a score from 1-5, depending on confidence of the tumor status (Table 1). 48% of these reviewed cases were included in the study (scores of 4 or 5) and 52% were excluded as undetermined (score of 3) or metastatic (score of 2 or 1). Tumors in both the undetermined and metastatic categories without TTF-1 staining could move to the primary category if a positive stain were provided in the future. 77% of the original study set of 115 cases were considered to have primary lung tumors and were included in analysis. Data was generated for undetermined or metastatic cases, but they will not be included in the statistical analysis. 26% of primary lung tumors were squamous cell carcinoma, 21% were adenocarcinoma, 21% were small cell carcinoma, 15% were mixed adenocarcinoma/squamous cell carcinoma and 17% were other histologies. 73% of the undetermined lung tumors and 81% of metastases were adenocarcinoma (Table 2). Assay data is presented for the categories of “All Cases”, which includes cases with any pathology review score, “Scores 4 and 5”, “Score 3”, and “Scores 1 and 2”.

Task 1: To determine the mutational spectra of the p53 tumor suppressor gene in paired, non-synchronous breast and secondary lung tumors in women. (Months 1-12)

a. Extract DNA from slides of breast and lung tumor tissue from 220 case and 123 control tumors from the Swedish Cancer Registry.
b. Sequence DNA extracted from samples using PCR amplification and the Affymetrix microarray system, including 20% repeated for quality control.
c. Analysis of sequence data based on radiotherapy and smoking status.

The first task for this project is to extract DNA from breast and lung tumor tissue and to use extracted DNA in the Affymetrix microarray system to detect mutations in the p53 gene. We received 110 cases and 123 controls from our collaborators at the Karolinska Institute in Stockholm, Sweden. Samples were logged in to the labortatory tissue repository database system, given a numerical identifier, and each slide was labeled with significant identifying information. DNA was extracted using a phenol-chloroform protocol. After extraction, samples were analyzed with a spectrophotometer to establish the concentration of DNA, normalized to 25ng/ul, and aliquoted to tubes for working stock and storage.

The Affymetrix Gene Chip system was used for mutational sequencing of exons 2-11 of the p53 gene. This technique has been used routinely for analysis of p53 mutations in a variety of populations. A multiplex PCR reaction amplifies exons 2-11, amplicons are fragmented and fluorescently labeled, and finally hybridized to probes on the GeneChip array using the Hybridization Station. The relative binding of template DNA to each probe in the array is determined with a laser scanner and evaluated with software that uses algorithmic analysis to give a numerical score for p53 mutations. The p53 multiplex amplification of DNA from tissue in formalin fixed, paraffin embedded blocks is widely accepted as problematic, especially for the largest exons. In this sample set, amplification of 368bp exon 4 was achieved only in about 30% of samples, however, the infrequency of published mutations in exon 4 makes amplification of this exon a low priority.

297 samples were successfully assayed for p53 mutations, with either a single PCR using the primers provided by Affymetrix or a nested strategy if the initial amplification was low, using primers just outside the Affymetrix primer sequences. Validation of the nested strategy was done by using the nested protocol to amplify 25 samples that had been originally amplified using the Affymetrix primers. After hybridization of these samples to the p53 GenChip, results were compared and found to be concordant. Exons in all study samples with GeneChip scores of 15 or higher were reamplified by PCR and sequenced using the MegaBace capillary sequencing system or repeated in the GeneChip assay to confirm the mutation.

Of cases with pathology scores of 4 or 5, those considered primary, 4 breast tumors had mutations and 15 lung tumors had mutations. Mutations in breast tumors included g>a changes, transition mutations, and missense mutations. Mutations in lung tumors were mostly a>g, t>c, and c>t changes, and missense mutations. 4 controls had mutations (Table 3).

Task 2: To determine ERA expression in paired, non-synchronous breast and secondary lung tumors in women and to establish primary tumor status of lung tumor tissue. (Months 13-24)

a. Perform immunohistochemical assays using ERA antibodies on breast and lung tumor tissue slides from 110 cases and 123 controls from the Swedish Cancer Registry.
b. Analysis of slide staining.

The second task for this project is to use immunohistochemistry to determine the ER alpha status of the breast and lung tumors and to establish the primary tumor status of the lung samples. 5 micron slides obtained from the tumor blocks were stained for ERA expression using ERA monoclonal antibody F-10 from Santa Cruz Biotechnology (Santa Cruz, CA), which recognizes the carboxy terminus of the

receptor protein. Citrate acid buffer was used for antigen retrieval, the antibody was used at 1:25 dilution for 1 hour at room temperature, followed by the StriAveGen Multilink Kit, staining with diaminobenzidine chromogen solution (DAB), and counterstaining with hematoxylin (all reagents from Biogenex; San Ramon, CA). Slides were examined by microscope for the presence of ERA staining and compared to the positive and negative control slides for each experiment. Determination of positive or negative expression status was made using the Allred scoring system, where numerical scores from 0-5 for proportion of tumor stained and 0-3 for intensity of staining are added for a final score; two or higher is considered positive for ER expression (76). All slides were double read by a pathologist and 20% were repeated for quality control.

Optimization studies were done to establish the correct dilution of the antibody for our protocol and to identify positive and negative control tissues. 117 control breast tumors, 110 breast case tumors, and 105 case lung tumors were stained for ERA. 81% of controls, 74% of case breast tumors, and 10% of case lung tumors were positive for ERA (Table 4).

Task 3: To determine methylation status of *GSTP1, p16, BRCA1, ER, 06MGMT,* and *cyclinD2* in breast and secondary lung tumors in women. (Months 25-36)

a. Perform PCR-based methylation assays on DNA extracted from 110 case and 123 control Swedish Cancer Registry samples, including 20% repeats for quality control.
b. Analysis of methylation patterns between breast and secondary lung tumors.

The third task for this project is to determine the methylation status of a panel of genes in breast and lung tumor tissue. Due to difficulties with quality control, only assays for p16 and Ecad were completed within the timeframe of this project. The other genes proposed will be investigated in this study set by other researchers in the lab. DNA extracted from tumor slides is subjected to bisulfite treatment, which results in the deamination of unmethylated cytosines. Deaminated cytosines become uracils, which are recognized as thymines by the Taq polymerase used in PCR. PCR is then performed using primers that differentiate between the methylated sequences and the unmethylated sequences, where thymines are substituted for cytosines.

A real-time PCR assay was used to detect methylation on the Taqman 7900 (Applied Biosystems), according to the method of Jeronimo et al. (84). Modified DNA is used as template with specific primers and probes corresponding to the methylated sequence. Primers and probes were designed by Applied Biosystems Assay-by-Design product. B-actin PCR was run first as a control to verify the presence of DNA after the modification. PCRs for p16 and Ecad were done in duplicate after b-actin and if all results were negative, the sample was re-modified and PCR was repeated. If results were still negative, the sample was excluded from the analysis. Samples with two positive results for p16 or Ecad were immediately called positive, while samples with only one or unclear results were repeated and considered positive if two out of three PCR results were positive.

279 samples have been analyzed for methylation of the promoter regions of p16 and Ecad. 2% of control tumors, 4% of case breast tumors, and 15% of case lung tumors were methylated for p16. 10% of case lung tumors were methylated for Ecad (Table 5).

Table 1. Explanation of pathology review scores.

Pathology Review Score	Explanation
1	definitely metastatic
2	probably metastatic; could be primary with positive TTF-1 stain
3	undecided; could be primary with positive TTF-1 stain
4	probably primary; different morphology in breast and lung, but would be stronger with a positive TTF-1
5	definitely primary; different morphology or positive TTF-1 stain

Table 2. Breakdown of histology of case lung tumors.

Primary (scores 4,5)		
	n	%
Adenocarcinoma	18	21
Squamous cell carcinoma	23	26
Adeno/squamous cell carcinoma	13	15
Bronchioalveolar carcinoma	10	11
Squamous cell carcinoma/sarcomatoid	1	1
Mucinous adenocarcinoma	2	2
Large cell	3	3
Small cell	18	21
Total	87	
Undetermined (scores 3)		
	n	%
Adenocarcinoma	8	73
Squamous cell carcinoma		0
Adeno/squamous cell carcinoma	3	27
Bronchioalveolar carcinoma		0
Squamous cell carcinoma/sarcomatoid		0
Mucinous adenocarcinoma		0
Large cell		0
Small cell		0
Total	11	
Metastases (scores 1,2)		
	n	%
Adenocarcinoma	13	81
Squamous cell carcinoma		0
Adeno/squamous cell carcinoma		0
Bronchioalveolar	1	6
Squamous cell carcinoma/sarcomatoid		0
Mucinous adenocarcinoma	2	13
Large cell		0
Small cell		0
Total	16	

Table 3. Summary of p53 mutations.

p53	control		all case breast[1]		all case lung		Scores 4, 5 prime case breast[2]		Scores 4, 5 prime case lung		Score 3 case breast[3]		Score 3 case lung		Score 1,2 case breast[4]		Score 1,2 case lung	
	n=99	%	n=100	%	n=98	%	n=73	%	n=73	%	n=9	%	n=11	%	n=16	%	n=14	%
wild type	95	96	93	93	78	80	69	95	58	79	8	73	9	82	14	88	11	79
any mutation	4	4	7	7	20	20	4	5.5	15	21	1	27	2	18	2	12	3	21
mutation type	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
ga	0	0	0	0	2	10	0	0	2	13	0	0	0	0	0	0	0	0
ag	1	25	4	40	5	25	3	75	4	27	1	100	0	0	0	0	1	33
ct	0	0	1	10	4	20	0	0	3	20	0	0	0	0	1	50	1	33
gc	2	50	1	10	3	15	0	0	1	6.7	0	0	1	50	1	50	1	33
tc	1	25	1	10	3	15	1	25	3	20	0	0	0	0	0	0	0	0
ac	0	0	0	0	1	5	0	0	0	0	0	0	1	50	0	0	0	0
ca	0	0	0	0	1	5	0	0	1	6.7	0	0	0	0	0	0	0	0
gt	0	0	0	0	1	5	0	0	1	6.7	0	0	0	0	0	0	0	0
transition	3	75	7	100	13	65	4	100	8	53	1	100	2	100	2	100	3	100
transversion	1	25	0	0	7	35	0	0	7	47	0	0	0	0	0	0	0	0
Mutation effect	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
missense	2	50	6	86	15	75	3	75	12	80	1	100	1	50	2	100	2	67
nonsense	1	25	1	14	3	15	1	25	2	13	0	0	0	0	0	0	1	33
silent	1	25	0	0	2	10	0	0	1	6.7	0	0	1	50	0	0	0	0
Mutation region	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
L2L3 loop	0	0	2	29	8	40	0	0	5	33	0	0	1	50	0	0	2	67
DNA binding	0	0	3	43	3	15	2	50	3	20	0	0	0	0	1	50	0	0
ECR	4	100	6	86	12	60	3	75	8	53	1	100	1	50	2	100	3	100
Exon	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
exon 5-9	4	100	7	100	20	100	4	100	15	100	1	100	2	100	2	100	3	100
5	3	75	0	0	7	35	0	0	5	33	0	0	0	0	0	0	2	67
6	0	0	0	0	3	15	0	0	2	13	0	0	1	50	0	0	0	0
7	0	0	3	43	4	20	1	25	3	20	0	0	0	0	2	100	1	33
8	1	25	3	43	5	25	2	50	4	27	1	100	1	50	0	0	0	0
9	0	0	1	14	1	1	1	25	1	6.7	0	0	0	0	0	0	0	0

1- all cases with tissue available idenitifed in the Swedish Cancer Registry. 2- only cases with scores of 4 or 5 in the pathology review. 3- only cases with a score of 3 in the pathology review. 4- only cases with scores of 1 or 2 in the pathology review.

Table 4. Summary of ERA results.

ER alpha	control		all case breast[1]		all case lung		Scores 4 and 5 prime case breast[2]		Scores 4 and 5 prime case lung		Score 3 case breast[3]		Score 3 case lung		Score 1,2 case breast[4]		Score 1,2 case lung	
Status	n=117	%	n=110	%	n=105	%	n=82	%	n=81	%	n=10	%	n=11	%	n=16	%	n=13	%
positive	95	81	81	74	10	10	60	73	2	2	7	70	3	27	12	75	5	38
negative	22	19	29	26	95	90	22	27	79	98	3	30	8	73	4	25	8	62
Allred score																		
0	22	19	29	26	95	90	22	27	79	98	3	30	8	73	4	25	8	62
2	0	0	8	7.3	4	4	6	7	1	1	0	0	1	9	2	13	2	14
3	1	1	4	3.6	0	0	0	0	0	0	2	20	0	0	2	13	0	0
4	6	5	8	7.3	3	3	6	7	0	0	2	20	2	18	0	0	1	8
5	12	10	12	11	1	1	10	12	0	0	1	10	0	0	1	6	1	8
6	14	12	11	10	0	0	8	10	0	0	1	10	0	0	1	6	0	0
7	18	15	15	14	0	0	13	16	0	0	1	10	0	0	0	0	0	0
8	44	38	23	21	2	2	17	21	1	1	0	0	0	0	6	37	1	8

1- all cases with tissue available idenitifed in the Swedish Cancer Registry. 2- only cases with scores of 4 or 5 in the pathology review. 3- only cases with a score of 3 in the pathology review. 4- only cases with scores of 1 or 2 in the pathology review.

Table 5. Summary of methylation data.

	control		**all case breast[1]**		**all case lung**		**Scores 4, 5 prime case breast[2]**		**Scores 4, 5 prime case lung**		**Score 3 case breast[3]**		**Score 3 case lung**		**Score 1,2 case breast[4]**		**Score 1,2 case lung**	
methylation	n=105	%	n=92	%	n= 80	%	n=70	%	n=61	%	n=8	%	n=9	%	n=12	%	n=11	%
any	2	2	4	4	17	21	4	6	14	23	0	0	3	33	0	0	0	0
p16	2	2	4	4	12	15	4	6	9	15	0	0	3	33	0	0	0	0
ecad	0	0	0	0	8	10	0	0	7	11	0	0	1	11	0	0	0	0
both	0	0	0	0	3	4	0	0	2	3	0	0	1	11	0	0	0	0

1- all cases with tissue available idenitifed in the Swedish Cancer Registry. 2- only cases with scores of 4 or 5 in the pathology review. 3- only cases with a score of 3 in the pathology review. 4- only cases with scores of 1 or 2 in the pathology review.

Key Research Accomplishments and Training (2004-2005)

- 343 tumors received, recorded, and labeled
- 343 tumors extracted for DNA
- 297 tumors analyzed for p53 mutations by Affymetrix Gene Chip

- 235 tumors stained for estrogen receptor alpha
- 277 tumors examined for *p16* and *Ecad* methylation
- Attendance at 2005 AACR Lung Pathogenesis meeting
- Attendance at 2005 ERA of Hope meeting
- Attendance at weekly Tumor Biology Program Journal Club and Tumor Biology Data meeting seminars
- Attendance at bimonthly lab meetings and weekly student meeting with Dr. Shields

Reportable Outcomes

-Abstract presented at Lombardi Cancer Center Research Fair 2005
Georgetown University, Washington, DC
-Data presented at the Tumor Biology Program Data Meeting 2004
Georgetown University, Washington, DC
-Poster presented at 2005 AACR Lung Pathogenesis Meeting, San Diego, CA
-Scholar-in-Training Award, AACR Lung Pathogenesis Meeting, San Diego, CA

Conclusions

Important questions have risen in the past year of work on this project, namely the verification of primary status of the case lung tumors and the quality of the methylation assay results. Establishment of the panel of methylation assays was challenging and only two of the six assays were completed validated with quality controls. However, these two assays alone suggest a role for methylation in radiation-induced tumors and the additional assays may provide further evidence of this role in the larger study that this project is a part of. The development of alternate methods for multiplex amplification of the p53 gene from samples with low quality or low quantity DNA, identified by weak PCR results upon visualization by gel electrophoresis, has been useful for other studies in our lab and will have a large impact on the study of which this project is a subset. Data generation for this project is complete and work has moved on to statistical analysis, which will be done in cooperation with our colleagues at the Karolinska Insistutet in Stockholm, Sweden.

There is significant evidence that indicates an increased risk for developing secondary lung cancer in women treated with radiotherapy for breast cancer and an additional risk for women who smoke. It is important to determine molecular markers for the susceptibility of breast cancer patients to lung cancer to improve information for treatment decisions. Markers in lung cancer may correlate with cancers that develop from radiotherapy alone, in combination with cigarette smoking, or with smoking alone. It is also important to examine the possibility that a particular predisposition, unrelated to any significant family history, has made these subjects more susceptible to different exposures, resulting in multiple primary tumors. It may be possible to define a unique subpopulation sensitive to hormonal risk factors for breast and/or lung cancer or to an alternate mechanism that could cause susceptibility to breast and lung cancer. Our hypothesis that p53 mutation spectra, methylation status, and ER expression are associated with risk of lung cancer following breast cancer could only be partially addressed by this project due to low sample size. Most subset analysis, regarding exposure to radiotherapy and smoking, was impossible. As this study continues, it will accumulate as many as 600 cases and the hypotheses will be more confidently assessed, providing information that may be useful in defining subpopulations sensitive to radiation therapy.

Women receiving radiation therapy following surgical treatment for breast cancer are believed to have improved disease free survival. For overall survival, however, benefits of radiotherapy have been more difficult to prove, especially for older women or those with a good prognosis. With information on increased risks based on radiation dose, breast cancer markers, history of smoking, and other factors, women can make more informed decisions about their breast cancer treatment. For women who elect to undergo radiotherapy, further screening and prevention methods may be devised. Women who smoke may be compelled by this additional risk information to quit smoking before radiotherapy. The degree of risk examined in this study is already considered to be at a level of importance similar to other risks of concern. The study that this project is a part of will be significant because it is larger than previous studies and can provide new data about radiation carcinogenesis and interactions with smoking through the use of molecular markers.

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