Molecular Cytogenetic Characterization Of Cell Lines Lacking Expression Of Interferon Regulatory Factor-1 on Chromosome 5

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MOLECULAR CYTOGENETIC CHARACTERIZATION OF CELL LINES LACKING EXPRESSION OF INTERFERON REGULATORY FACTOR – 1 ON CHROMOSOME 5

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology and Chemistry at Carroll College, Helena, Montana

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This thesis for honors recognition has been approved for the Department of Biology and Chemistry by:

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The molecular cytogenetic techniques of fluorescence *in situ* hybridization (FISH) and karyotyping by means of GTG-banding were used to determine if a chromosomal abnormality could be detected in cell lines lacking expression of the Interferon Regulatory Factor -1 gene (IRF-1). It is believed that IRF-1 plays an important role in the biological immune response leading to the presence of Human Leukocyte Antigens (HLA) class II. These molecules are important indicators for some cancerous cells. Without HLA class II expression a cell may avoid an immune attack by T-cell receptors of CD4+ cells, thus the cell may continue on its cancerous course. Four malignant cell lines that lacked IRF-1 expression (WERI, Y-79, H1734, and H322) were examined by focusing on the long (q) arm of chromosome 5 at band region q31, the IRF-1 gene locus. The results for each cell line did not indicate a deletion at 5q31. However, multiple but inconsistent abnormalities could be characterized, and future analysis is recommended.
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INTRODUCTION

Cancer - how can we approach such a dreadful disease? About one out of every four human deaths in the United States is due to cancer (CDC, 1997). Cancer is essentially a genetic disease (Meltzer and Trent, 1998). This does not mean that all cancers are inheritable. It simply means that there are problems at the gene level that lead to the expression of the disease. There are numerous genetic mistakes that can occur in the trillions of cells that make up the human body. Some of the genetic problems that lead to cancer involve one or more of the following: proto-oncogenes, tumor suppressor genes, repair genes, or genes involved in the immune response. These genes may be altered or lost by means of chromosomal abnormalities or mutations. The end product is an insubordinate cell that grows and divides at will, a cancerous cell.

Chromosomal abnormalities are classified according to the extent in which they differ from the normal chromosomal arrangement. There are two types of chromosomal abnormalities. The first type of chromosomal abnormality results in a numerical change in the total number of chromosomes, for example aneuploidy. This results from the loss or gain of a specific chromosome. The second type of chromosomal abnormality is a structural change that involves chromosomal breakage, like an inversion or translocation (Strachen and Read, 1996). Such chromosomal abnormalities may be referred to as chromosomal mutations. However, for this paper, a mutation will be defined more strictly. A mutation will be considered a loss or substitution in the nucleic acid sequence causing a change within a single gene located at a particular chromosomal locus, hence a point mutation.

Either type of alteration can result in the development of cancer. Most cancers result from somatic alterations and the accumulation of mutations. One of the most popular examples of cancer development is retinoblastoma. Retinoblastoma is a childhood disorder characterized by the development of malignant tumors in the retina.
before the age of six (Cavenee and White, 1995). This disease was studied by Knudson in 1971 and led to his two hit hypothesis (Cavenee and White, 1995). If one allele is mutated the normal allele will maintain order, but if both alleles mutate then retinoblastoma arises. In other words, in order for most cancers to fully develop it usually takes two or more mutations to develop within the same cell (Griffiths et al., 1993). This leads to the theory of clonal evolution. Clonal evolution is thought of as a multiple step process. First, a single cell undergoes a genetic mutation enabling it to divide when a normal cell would not. This abnormal cell divides, passing on its genetic changes to the next generation. Later, one of the descendental cells undergoes another mutation that further enables the cell to escape regulation of its cell cycle. Through this repetitive process of mutations, a deadly daughter cell may be produced with the ability to metastasize and colonize other organs of the body (Cavenee and White, 1995). A cancerous cell that has the ability to travel within the body and colonize other sites is very deadly and is referred to as a malignant tumor. A tumor that does not have the ability to travel is benign, but that does not mean it is not dangerous. A benign tumor in the brain can still cause death.

One way to study cancer focuses on the role genes play in controlling the cell cycle. The cell should be under the control of two growth pathways, one stimulatory, the other inhibitory. The former involves the proto-oncogenes which direct the cell to divide, whereas the latter involves the cessation of division by tumor suppressor genes (Weinberg, 1996). Both of these pathways are structurally similar with regard to the following procession: signal molecules bind to membrane receptors, cytoplasmic proteins are released and cross the nuclear membrane, these proteins act as transcription factors and instruct the DNA to transcribe mRNA, finally the message is translated into proteins that promote or impede cell division (Weinberg, 1996). If a genetic alteration such as a chromosomal abnormality or mutation disrupts either pathway then cancer may develop.
If the tumor suppressor genes or proto-oncogenes are altered then a cancerous problem is possible. The former often follows Knudson's two hit hypothesis (Personal Communication, Dr. Haag). For example, if both alleles in a tumor suppressor gene are mutated then its protein product will not function properly. If the protein product is a cytoplasmic relay protein then this particular cell cannot be directed to cease cell division by an extracellular inhibitory molecule. With the cessation of outside control, this cell may have the ability to proliferate without inhibition. It could be considered cancerous.

As for proto-oncogenes, these usually become insubordinate divide signaling genes called oncogenes by means of a single chromosomal abnormality and / or clonal evolution (Personal Communication, Dr. Haag). A good example is chronic myelogenous leukemia (CML). CML is the result of the translocation between one chromosome 9 and one chromosome 22 (CML, 1998). A proto-oncogene on chromosome 9 called c-abl is moved to the middle of a gene on chromosome 22 known as bcr. The resulting chromosome is called the Philadelphia chromosome, and the two genes form a hybrid bcr-abl gene that produces an abnormal protein, increasing the rate of mitosis (CML, 1998). The abnormal gene product is a tyrosine kinase (Strachan and Read, 1996). Clonal evolution may contribute to the activity of this tyrosine kinase by further mutating the descendant cells so the rate of mitosis rises sharply (CML, 1998).

Cancer can also arise when a repair gene is damaged. These genes known more specifically as nucleotide excision repair (NER) genes are responsible for producing enzymes that find and eliminate any damaged DNA (Wood, 1995). Mistakes in the sequence of DNA can occur at any time. Without functional NER genes, the body is more prone to have such mistakes persist through replication. People with damaged repair genes are more mutation-prone and have a higher risk of developing cancer (Hamilton, 1995). This higher risk of cancer by non-functional NER genes can be traced back to the theory of clonal evolution. More mutations can accumulate when NER genes
are not fully functional. Thus cancer will be more common in individuals with this problem.

Another pathway to cancer can exist with immune response complications. When a normal cell undergoes a genetic alteration and becomes cancerous, some of its surface antigens change (Schindler, 1993). The immune system can recognize the altered cell antigens and destroy the cell. Cancer may arise when the cell is not flagged for destruction, and it continues to divide. Such a failure in the immune response will be the focus of this paper. The hypothesis will suggest a genetic problem in the biological immune response leading to the lack of tumor antigen presentation on cancerous cells. This was studied through the use of two cytogenetic methods of cancer cell analysis: fluorescent *in situ* hybridization (FISH) and GTG-banding for karyotype analysis.

FISH is a very popular molecular cytogenetic technique. It involves the hybridization of DNA probes to a complementary target sequence in order to answer genetically related questions (Dyer and Meyne, 1991). FISH uses single stranded DNA probes marked with a fluorophore, a chemical group that is capable of fluorescing (Strachan and Read, 1996). The fluorophore may be attached to the DNA sequence either directly or indirectly. Commonly digoxygenin or biotin is used as a label and incorporated into the probe's nucleotide sequence. An antibody attaches to this nucleotide label, and a fluorophore combines with the antibody. With the use of a fluorescence microscope and the appropriate filters, the fluorophore can be observed, thus indicating the presence and position of the tagged nucleotide sequence of interest.

The fluorescent probe is hybridized to a double stranded DNA target. In order for hybridization to occur both the probe mixture and the DNA target must be denatured. During denaturation the hydrogen bonds holding the two complimentary DNA strands together are temporarily disrupted. The single stranded probe and target DNA hybridize as anti-parallel, complimentary sequences. The non-binding excess is then washed away.
Hybridization may be done with both interphase and metaphase chromosomes, but this may depend on the type of probe used.

There are three general FISH probes that are used in cytogenetic analysis. The first one is a centromeric probe. This probe recognizes repetitive sequences located on all chromosomes; they are used in both metaphase and interphase preparations (McGavran, 1996). The second type of probe is a locus specific probe. These probes hybridize to specific regions of a particular chromosomal arm. A chromosome has two different arms; one is called p for 'petit' while the other is q for 'grand' (Mueller and Young, 1995). These probes will be complimentary to a sequence found in one region of the chromosomal arm in question. Thus, they are good indicators for critical gene regions where a deletion may be suspected. They too can be used in both interphase and metaphase cells (McGavran, 1996). Finally, there is the whole chromosome paint probe. As the name implies this probe hybridizes to fluoresce an entire chromosome. It is made up of a number of smaller overlapping probe fragments (Strachan and Read, 1996). It is most useful in metaphase spreads because it can easily show translocations and insertions.

FISH has proved to be a valuable cytogenetic technique for a number of reasons. First, it is a very speedy process. FISH results can be obtained overnight. Second, it may be applied to both interphase and metaphase cells. This is an advantage since other techniques, such as GTG-banding, require nice, well-spread metaphases only. Finally, FISH can reveal a myriad of different genetic defects such as inversions, translocations, aneuploidy, polyploidy and deletions with its beautiful fluorescent signals. Nonetheless, there are some drawbacks to FISH. The reliability of the probe must be questioned for clinical analysis. Currently, there are no set of standards for evaluation of FISH probes by the Food and Drug Administration (Fox, 1996). However, the FDA has nothing to do with FISH as a research tool. The only true drawback to FISH in the laboratory is that a
FISH probe may not detect microdeletions in the DNA sequence. Microdeletions may be defined at two different levels. Microdeletions on the nucleic acid level involve deletions of a few (1-10) base pairs. Microdeletions can also be thought of in the cytogenetic sense as a deletion that cannot be detected by banding analysis – less than 4 x 10^6 base pairs (Personal Communication, Dr. Haag). Because FISH probes can be quite large, 200 kilobase pairs, a few base pair deletions will not prevent the probe from binding. Using the definition of microdeletion as a few base pair deletions FISH will not be very practical. Thus, in the cytogenetic sense, FISH is extremely practical in locating deletions that cannot be detected by banding. However, in the case of a microdeletion of only a few base pairs, FISH will not be an adequate indicator. Nonetheless, its speed and wide range of application make fluorescence in situ hybridation a valuable tool for looking at some of the genetic diseases.

The second technique used in this project was karyotyping by means of GTG-banding. A band is considered to be an area on the chromosome made distinguishable by its light or dark appearance (Heim and Mitelman, 1987). There are numerous banding methods that produce different light and dark patterns on the chromosomes. The treatments usually involve denaturation and/or enzymatic digestion of chromatin and the use of a DNA-specific dye (Strachan and Read, 1996). GTG-banding uses the enzyme trypsin to partially digest the chromatin, and a stain called Giemsa is applied. The Giemsa stain has an affinity for a particular type of chromosomal packaging, scaffold attachment regions (SARs). A SAR is made of nonhistone acidic proteins which attach to regions of chromatin fibers that are usually greater than 65% adenine/thymine sequences (Strachan and Read, 1996). The scaffold arrangement of chromatin fibers creates the highly dense chromosomal appearance seen in dividing cells. The differential stain is applied to metaphase chromosomes so that variations in the extent of condensation can be identified and analyzed. The stained chromosomes are
photographed and arranged on paper as pairs in a set that is known as a karyotype. A karyotype also refers to the total number of chromosomes and specific sex chromosomes present in a cell (Strachan and Read, 1996).

The benefit of the karyotype is that chromosomal defects such as inversions, translocations, and large deletions can be observed. Unfortunately, this process is slow. Nicely elongated, well-spread chromosomal metaphases must be prepared and stained. Photographs must be taken of the stained spread, the film must be developed and the individual chromosomes cut out. This is a tedious process that takes a lot of skill and a little luck, but in the end it produces a reasonably accurate analysis to the trained eye.

The GTG-banding and FISH techniques discussed were used to analyze four different tumor cell lines. The cell lines are continuous meaning that they are long-term cell cultures that may be maintained indefinitely in vitro. This immortality is common in tumor cell lines (Lee, 1991). Normal cell lines go through a life cycle that starts off in a shocked lag phase, then explodes into a phase of exponential growth and after about 50 passages growth starts to decrease and the cells begin to die. A passage is when a portion of the cell population is split apart from the rest and transferred from one flask to another with fresh medium. Due to their genetic abnormalities, tumor cell lines do not follow a normal cell cycle in vitro. If the cell line is continuously split then the cell line will grow and divide forever.

There are some advantages and disadvantages with a continuous cell line. The primary advantage is that a large population of cells is available that may be examined and re-examined (Lee, 1991). Such continuous examination is good since multiple laboratories can study the same cell line and confirm or dispute results. However, a continuous cell line will evolve and chromosomal changes will take place, especially with a tumor cell line. This idea was discussed with clonal evolution and the progressive gain of mutations in descendent cells. Thus, to insure that any chromosomal
abnormalities represent in vivo conditions, a short-term (mortal) cell line is preferred
(Heim and Mitelman, 1987). In addition, extreme caution must be taken with any cell
line to prevent cross contamination with another. The experimenter must be very careful
when pipetting, feeding and splitting a cell line. The safest precaution is to work with
only one cell line at a time and to keep all cell lines separate. A continuous tumor cell
line may be beneficial in the analysis of cancer and the immune response, but extreme
cauion and recognition of its limitations must be maintained.

There are two types of cell lines, monolayer and suspension. A cell line that is
characterized as a monolayer adheres to the bottom of the culture flask, in which it is
grown, whereas a suspension culture grows free-floating in the medium. This experiment
used two suspension (Y-79 and WERI) and two monolayer cell lines (H322 and H1734)
that were all continuous. The cytogenetic techniques of FISH and GTG-banding for
caryotyping were used to analyze these cell lines for a chromosomal abnormality or
mutation in the immune response pathway.

The experimental approach of this project was to use the two cytogenetic
techniques (discussed above) to analyze cell lines lacking expression of interferon
regulatory factor-1 (IRF-1). The IRF-1 gene is thought to play a role in the immune
response pathway. It appears that this gene is required for expression of Human
Leukocyte Antigen (HLA) class II molecules. The HLA class II molecules present a
protein/peptide complex on the surface of the cell that can be recognized by CD4+ helper
T cells (Blanck, 1997). The helper T cells can trigger an immune response against the
antigen by releasing a cytokine called interferon (IFN) gamma. The IFN gamma may
activate macrophages, which will become cytotoxic to any cell in the immediate vicinity,
including the tumor cell (Benjamini et al., 1996).
However, there is a slight problem. It is thought that IFN gamma also has an indirect role in triggering the IRF-1 gene which in turn leads to HLA class II molecules on the surface of the cell (Blanck, 1997). Thus, how can HLA class II molecules trigger the release of IFN gamma from helper T-cells, if IFN gamma is required for HLA class II expression? Several solutions are feasible. One possibility, suggested by Dr. Blanck’s graduate student Donna Berry, is that professional antigen presenting molecules engulf dead tumor cells and express these tumor antigens on MHC class II molecules which would activate the immune response (Personal Communication, Donna Berry). Another possible solution is that IFN gamma is released by helper T-cells that recognize non-specific morphological changes in the tumor cell. This non-specific response would lead to IFN gamma release and the eventual HLA class II expression. This raises the question of why the macrophages are activated with this initial IFN gamma? The answer to the question remains concealed in the complexity of the relationship between the tumor and tumor associated macrophages, and cytokines other than just IFN gamma (Benjamini et
Overall, the focus of this project is on the IRF-1 gene. The four cell lines used in this experiment lack expression of this gene. This was determined by an electrophoretic mobility shift assay (EMSA). An EMSA utilizes the idea that if a protein inducer binds to a DNA binding region, its electrophoretic mobility will be reduced (Strachan and Read, 1996). Thus, the DNA binding region for the IRF-1 gene was analyzed to determine if a regulatory protein was binding and activating the gene or not. This method did not show any activation of the IRF-1 gene in the four cell lines studied. The objective was to determine whether this gene was present or moved in the genome of the non-expressive cell lines. The cytogenetic techniques of FISH and GTG-banded chromosome analysis were utilized to detect a deletion, translocation or inversion in the IRF-1 gene region. This particular gene is thought to be located on chromosome 5 at 5q31. Therefore, the chromosomal region 5q31 was the focal point of this molecular cytogenetic analysis.
MATERIALS AND METHODS

Tissue Culture

All four cell lines were grown in a CO₂ chamber at 37°C. They were bathed in a medium made from 1640 Roswell Park Memorial Institute (RPMI) media formula with L-glutamine, 10% Fetal Bovine Serum, and antibiotic-antimycotic (Life Technologies). The medium contains a phenol red pH indicator. This indicator appears red at an alkaline pH, golden yellow at a neutral pH, and yellow at an acidic pH (Babu and Verma, 1995). All four cell lines were frequently observed with the use of an inverted microscope. They were carefully handled under aseptic conditions. These conditions involved all work to be done in a laminar flow hood, the use of latex gloves, the use of an electric pipette with sterile pipettes and scrub down of the hood with 70% reagent alcohol before and after use.

Werî (retinoblastoma cell line)

This is a suspension cell line that was split 1:2, 1:5 or 1:10 according to need. A split of 1:2 means that one half of the total number of cells was kept and the other half discarded. All discarded cells were placed in a 10% solution of bleach. The cells were grown in either a Corning 25 cm² or 75 cm² tissue flask. The smaller tissue flasks were maintained with 5 ml of medium, whereas 50 ml was used in the larger flasks.

Y79 (retinoblastoma cell line)

This is also a suspension cell line with some cells adhering loosely to the flask. They were easily detached by pipetting, and they were split 1:5 or 1:10 about every four days. The cells were grown in either a Corning 25 cm² or 75 cm² tissue flask.

H1734 (non-small cell lung carcinoma cell line)

This is a monolayer culture that required the use of fresh trypsin in order to loosen the adhesion of the cells to the bottom of the flask (see trypsin treatment). The cells were usually split 1:2 about every ten days. The cells were maintained in Corning 25 cm²
tissue flasks.

**H322 (non-small cell lung carcinoma cell line)**

This is also a monolayer cell line that required trypsin treatment (see below). These cells were split 1:5 about every five days. They were maintained in Corning 25 cm² tissue flasks.

**Trypsin Treatment**

The cell medium was discarded into a 10% bleach solution. The cells were washed twice in Hank's Balanced Salt Solution (HBSS). The HBSS wash was kept because a few cells would sluff off into this solution. Then a small amount (1-2 ml) of GIBCO EDTA trypsin was added - just enough to cover the bottom of the flask. The flask was placed in the 37°C incubator for about 2 minutes. The flask was then pounded forcefully against the palm of the hand. The cells were observed under the inverted microscope to make sure of their detachment. Then the cells were mixed by pipetting and either split or placed in centrifuge tubes for harvest or freezing.

**Cryogenics**

*Freezing* (All four cell lines were frozen using this procedure.)

A freezing medium was made consisting of the cell medium (mentioned above) and 10% Dimethyl Sulfoxide. The cells were removed from their flask (trypsinated if necessary) and placed in 15 ml centrifuge tubes (medium was usually added to bring volume up to about 10 ml). The cells were centrifuged for 10 minutes at 900 rpm. The supernatant was discarded and the pellet of cells was resuspended in about 1.5 ml of freezing medium. The solution of freezing medium and cells was transferred to a freezing vial. The vial of cells was placed in a - 4° C freezer for 30 minutes. Then the vial was transferred to a - 60° C freezer for storage.

*Thawing*

The frozen vial was shaken by hand in a 37° C water bath until it was partially
thawed. This solution was pipetted into a 15 ml centrifuge tube with 10 ml of warm medium. This tube was centrifuged for 10 minutes at 900 rpm. Finally, the supernatant was discarded and the pellet of cells was resuspended in culture.

**Harvesting**

This procedure was adopted from Shodair Hospital’s protocol for cell preparation. Colcemid (5 µg/ml) was added to the culture flask so that the concentration of colcemid was 0.1 µg/ml. Colcemid (10 µg/ml) was also used. This gave a respective concentration equal to 0.2 µg/ml. The flasks were incubated at 37°C in a CO₂ incubator (time varied). After the desired exposure time to colcemid, the medium was poured into a 15 ml centrifuge tube. The flask was washed two times with about 2 ml HBSS and the washes were added to the centrifuge tube. If the cell line was a monolayer then it was trypsinized at this point (see trypsin treatment), and then the cells were added to the centrifuge tube. Medium was then added to bring the final volume up to about 12 ml. The addition of medium is especially important for the trypsinized cells. It is important because the fetal bovine serum found in the medium inactivates trypsin so it no longer affects the cells. The tubes were then centrifuged for 10 minutes at 900 rpm. The supernatant was discarded and the pellet was resuspended in 0.5 ml of warmed 37°C hypotonic solution [3 different hypotonic solutions were used: a) 3:1 0.038 M KCl + 0.4% NaCitrate b) 0.057 M KCl c) 0.075 M KCl]. The cells in the hypotonic solution were left at room temperature for either 10 or 15 minutes. These cells were periodically pipetted during this time. They were then centrifuged for 10 minutes at 900 rpm. After removal from the centrifuge the supernatant was removed except for 0.5 ml left on the pellet. The pellet was resuspended in the 0.5 ml of hypotonic solution. Then a solution of 3:1 absolute methanol:glacial acetic acid fixative was added slowly. This required mixing by pipetting between small (2-3 drops) additions of fixative. The fixative was added until the total volume was about 4 to 5 ml. The cells were then centrifuged for 10
minutes at 900 rpm. The process of removing the supernatant (fixative solution) and resuspending the cells in fixative is repeated twice with a 10 minute centrifuge time in between. When it was time to add the final fixative solution to the cells, a fresh solution was prepared. (Note, it was not required to add the fixative slowly the second and third times, only when it was first introduced to the cells after hypo treatment.) Finally, the cell pellet was resuspended in the fresh fixative solution. The cells in the fix solution were then dropped onto slides. The slides could be observed with a phase contrast microscope, and any changes in the dropping procedure could be modified. For example, if there were not very many cells on the slide then more drops of cell/fix solution could be dropped.

*Slide Preparation*

Slides were prepared by dropping the fix/cell solution from a height of two feet or greater from a sterile glass pipette onto a slide. The slides (Fischer - premium) were usually placed in distilled H₂O at room temperature just before they were dropped. Various techniques were tried such as breathing on the slide, using cold slides, and dropping higher/lower.

*FISH protocol (for both locus specific & whole chromosome probes)*

If the slide was less that two weeks old it was first aged in 2XSSC for 30 min. at 37°C. Then it was dehydrated at room temperature in 70%, 85%, 95% ethanol solutions for 2 min each. This process solidifies the protein of the chromosome so when it is denatured the entire chromatin structure does not come apart.

The specimen slide was first denatured in 70% formamide/2XSSC at 73+/-1°C for 2 to 4.5 min. The dehydrated specimen slides were then placed in a series of 2 min ethanol washes (70%, 80%, 95%, and 100%) to maintain the denatured state of the specimen. Then 1µL probe (Vysis), 2 µL sterile H₂O and 7 µL Hybridization Buffer
(Vysis) were mixed in a small sterile vial. The probe mixture was denatured in a water bath at 73+/-1°C for 5 minutes. The denatured probe was then applied to the specimen target area (approximately 22 x 22 mm or half a slide). The probe DNA will hybridize to the denatured target specimen DNA at 37°C for 6-16 hours (overnight). A coverslip was placed over the probe/specimen and sealed with rubber cement to prevent dehydration. The rubber cemented slides were then placed in a rubbermaid container with wet paper towels. This container was then placed in the incubator overnight. The rubber cemented coverslip was removed the following day with tweezers and discarded. This was followed by a rapid wash consisting of 0.4X SSC/0.3% NP-40 at 73+/-1°C (2 min). Following this was another wash consisting of 2X SSC/0.1% NP-40 at ambient temperature (30 s). This was sometimes followed by a quick dip in sterile H₂O to prevent salt crystal formation on the slide due to the NP-40 wash. The probe hybridized slide was then allowed to sit, but it was not allowed to completely air dry. Finally, 10 uL of DAP II counterstain was applied to the target region and coverslip was added. The slide was then observed using a fluorescence microscope and appropriate filters.

**FISH Probes (from Vysis, Inc. Quality Assurance Certificates)**

**A) LSI 5q EGR-1 SO / D5S23 SG Lot# 9859 Expiration Date: 09-12-98**

This is a dual colored locus specific DNA probe that hybridizes to two regions on human chromosome 5. SpectrumOrange LSI 5q EGR-1 (200 kb) hybridizes to band 5q 31, the EGR-1 gene locus. SpectrumGreen LSI D5S23 (5p) (360-420 kb) hybridizes to band 5p15.2, locus D5S23.

**B) LSI CSF1R SO/D5S721:D5S23 SG Lot# 10191 Expiration Date: 09-19-98**

This is also a dual colored locus specific DNA probe that hybridizes to two regions on human chromosome 5. SpectrumOrange LSI CSF1R (140-180 kb) hybridizes to band 5q32. SpectrumGreen LSI D5S721:D5S23 (5p) (360-420 kb) hybridizes to band 5p 15.2, loci D5S721 and D5S23.
This is a whole chromosome paint DNA probe that hybridizes to the 5p arm, 5q arm and the centromere of human chromosome 5.

**GTG-banding protocol**

The specimen slide was baked in a 60 °C oven overnight. The baked slide was then cooled to room temperature. After it was cooled, it was placed in a 100 ml trypsin (DIFCO) solution for 20-35 s. The slide was then removed and quickly washed in two separate isotonic solutions. The slide was then placed in a solution of KaryoMAX Giemsa (improved R66 "Gurr" solution-buffer) stain for 2 min. Finally, the slide was removed, and the stain was washed off in sterile H₂O. The slide was then blown dry with compressed air and viewed under the microscope.

**Karyotyping**

A camera attached to a microscope was used to take pictures of well stained and widely spread metaphases (few crossovers) of interest. The film was then developed and prints were made in the black and white photo lab. The prints of the chromosome spreads were carefully cut out, and the task of identifying the chromosomes was pursued. Finally, the identified G-banded chromosomes were placed on a karyotype sheet using double stick tape.
RESULTS

The Vysis EGR-1 and CSF-1R probes are actually combination probes. They are dual colored meaning they have two locus specific probes with two different colors. One of the probes is shared by both EGR-1 and CSF-1R. This probe identifies chromosome 5 by hybridizing to the 5p15.2 region, and it fluoresces green. This green colored probe is not the probe of interest. It is simply a marker for chromosome 5. For example, if there is a deletion or translocation in the region of interest (5q31 or 5q32) then chromosome 5 will still be identified by the green signal.

The difference between EGR-1 and CSF-1R lies in their red signal. EGR-1's red signal is specific for region 5q31, while CSF-1R is specific for 5q32. These two different probes were selected because of their proximity to each other. If EGR-1's red signal is deleted and CSF-1R's red signal is not then the deletion can be narrowed down to the 5q31 region.

The FISH procedure will detect other genetic abnormalities of interest as well, such as a translocation or an inversion. A translocation would be detected if the red signal from either dual color probe was on a chromosome other than the one containing the green marker signal. This of course assumes that the green marker signal is not affected. An inversion could also be observed if the red signal appeared in a different location on the chromosomal arm than expected. Overall, the primary focus of this study was on the red signal of EGR-1 (Fig. 1) because this is approximately where the IRF-1 gene is located. Both of the marker and the gene are supposedly located in the banding region 5q31. Thus, any major problems with this region would be indicated with the EGR-1 probe.

Werí (retinoblastoma cell line)

Looking at the cell line results it appears that both the FISH locus specific probes
(EGR-1 (Fig. 2) and CSF-1R (Fig. 3)) indicate three green signals (5p15.2) and two red signals (5q31 and 5q32). This indicates that there is a duplicated 5p15.2 region. However, this is merely the green marker signal. The areas of interest, 5q31 and 5q32, appear normal with two red signals per metaphase spread. With a normal diploid metaphase spread, one would expect two signals for the two homologous chromosomes. Of course, some of the chromosomes may spread too far on the slide as a result of the dropping procedure. This may account for the six cells with only two green signals and the one cell with one red signal. The remaining marked chromosomes were blown farther away and were not counted in the metaphase spread.

FISH with the whole chromosome paint (WCP) (Fig. 4) for chromosome five indicated that there were two apparently whole chromosome fives and one partial five on the bottom of the q arm of another chromosome. The WCP and the locus specific EGR-1 were both conducted on the same slide and same metaphase spreads. First, the EGR-1 hybridization was completed and analyzed. This was followed by the second denaturation of the same chromosomes and rehybridization with the WCP. The results of two whole chromosome fives and one partial corresponded with the previous EGR-1 results of 3 green / 2 red. Thus, the extra 5p15.2 signal is the partial that is duplicated onto the bottom of the q arm of some other chromosome.

The karyotype by GTG-banding (Fig. 5) indicated there are a number of numerical and structural abnormalities. It appears that there are two chromosome fives, but one does look shorter than the other. Nonetheless, the short appearance of one of the five chromosomes is not due to a deleted 5q31 or 5q32 because these were normal according to the FISH results of EGR-1 and CSF-1R respectively.

The retinoblastoma suspension cell line liked to clump up and did not spread very well on the slides. A dozen harvests were attempted. The best harvest was done 10/30/97. FISH was not used on these slides until later due to lack of better slide
harvests.

**Y79 (retinoblastoma cell line)**

Both of the locus specific FISH probes indicated the same signal results. It appears that there is one green signal (5p15.2) and two red signals (5q31 and 5q32) - (Fig. 6). This is an indication of a deletion in the marker region 5p15.2. Nonetheless, the red signals of primary interest did not show any aberration in this cell line. Since there was no indication of a duplicated region in the Y79 line, the WCP #5 was not used.

The karyotype for this cell line (Fig. 7) indicated one normal appearing five and one visually abnormal five with a very short p arm. The short p arm on one of the five chromosomes corresponds with the FISH results. FISH showed a deletion in the 5p15.2 region which is reflected with the abnormally short p arm.

Specimen slides were obtained on this cell line early on in the project on 9/23/97.

**H1734 (non-small cell lung carcinoma cell line)**

Both of the FISH locus specific probes show a myriad of results, especially with the green marker probe. However, if one looks closely at the cell line results the red signals of interest are fairly normal appearing (Figs. 8 & 9). There are usually two red signals (may look a little white on the color copy). Thus, no major problem with the IRF-1 gene location can be observed.

The FISH WCP #5 (Fig. 10) was done on the same slide and metaphase spreads as used for EGR-1. Yet, no conclusive evidence can be seen in the mix of results obtained from this probe. The karyotype also leads to some confusion (Fig. 11). There are possibly four chromosomes that may be possible fives or partial fives. With the karyotype it is even more difficult to determine what is a chromosome five and what is not.
Specimen slides were used for both on 9/25/97 and 12/10/97.

H322 (non-small cell lung carcinoma cell line)

The locus specific probes gave the same results for this cell line. Both the EGR-1 (Fig. 12) and CSF-1R (Fig. 13) showed three green signals (5p15.2) and two red signals (5q31 and 5q32). This appears to be the same results found in the WERI cell line. Of course, this brings up the possibility of mislabeling or cross-contamination, but the WCP #5 answers this question.

FISH with the WCP #5 (Fig. 14) indicated two possible whole five chromosomes and one partial five chromosome, as in WERI. However, the key is the partial chromosome. The partial in this cell line is located on both the p arm and top of the q arm of another chromosome. This result is important because it refutes the possibility of contamination with the WERI cell line. WERI seems to have the same FISH results up to this point; however, the WCP #5 on WERI showed the partial five signal on the bottom of another chromosome's q arm.

The karyotype indicated (Fig. 15) that there are two ‘whole’ chromosome fives and an additional five segment. One of the ‘whole’ chromosome fives is shorter than the other one. There also seems to be a partial 5p arm and maybe some of the 5q arm as an additional chromosome.

Harvesting this NSCLC cell line was a problem due to the amount of cytoplasm that persistently remained on the slides. A stronger hypotonic solution was tried on 11/9/97. The stronger solution caused the cells to swell up more so that when the slides were dropped the chromosomes would spread with more force. This force was required in order to overcome the residual cytoplasm problem.
<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Weri</th>
<th>Y79</th>
<th>H1734</th>
<th>H322</th>
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<tr>
<td><strong>Lab #</strong></td>
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<td>97-537</td>
<td>97-538</td>
<td>97-539</td>
</tr>
<tr>
<td><strong>GTG-Banding Results (Karyotype)</strong></td>
<td>two 5 chromosomes and perhaps a partial</td>
<td>one normal 5 and one abnormal 5 with a very small p arm</td>
<td>possibly four 5-like chromosomes</td>
<td>two 5 chromosomes and perhaps a partial</td>
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<tr>
<td><strong>FISH: EGR-1 results</strong></td>
<td>Total Scored: 20 14 = 3 green / 2 red 6 = 2 green / 2 red 1 = 2 red</td>
<td>Total Scored: 23 23 = 1 green / 2 red</td>
<td>Total Scored: 10 4 = 5 green / 2 red 2 = 4 green / 2 red 1 = 5 green / 3 red 1 = 0 green / 2 red 1 = 1 green / 2 red 1 = 2 green / 1 red</td>
<td>Total Scored: 20 20 = 3 green / 2 red</td>
</tr>
<tr>
<td><strong>FISH: CSF-1R results</strong></td>
<td>Total Scored: 20 17 = 3 green / 2 red 2 = 2 green / 2 red 1 = 4 green / 3 red</td>
<td>Total Scored: 20 20 = 1 green / 2 red</td>
<td>Total Scored: 10 2 = 5 green / 2 red 2 = 3 green / 2 red 2 = 4 green / 2 red 2 = 0 green / 2 red 1 = 7 green / 4 red 1 = 1 green / 3 red</td>
<td>Total Scored: 20 19 = 3 green / 2 red 1 = 2 green / 2 red</td>
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<tr>
<td><strong>FISH: WCP #5 results</strong></td>
<td>(Analysis of same chromosome spreads as EGR-1) 2 'complete' chr. 5 1 partial chr. 5 The partial appears to be located at the bottom of another chromosome's q arm. It is the extra green signal (5p 15.2)</td>
<td>This was not used because there was no sign of a duplicated region of interest.</td>
<td>(Analysis of same chromosome spreads as EGR-1) Only 6 of 10 counted: 2 = 2 whole / 3 partial 1 = 1 whole / 3 partial 1 = 2 whole / 4 partial 1 = 3 whole / 4 partial 1 = 2 whole / 2 partial</td>
<td>(Analysis of same chromosome spreads as EGR-1) 2 'complete' chr. 5 1 partial chr. 5 The partial seems to be located on the p arm and top of the q arm of another chromosome. It is the extra green signal (5p 15.2)</td>
</tr>
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<td>Sample</td>
<td>Date</td>
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<td>Colcemid Time</td>
<td>Hypo Conc.</td>
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<td>---------------</td>
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<tr>
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<td>0.057 M KCl</td>
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<td>3.1 0.038 M KCl + 0.4 % NaCitrate</td>
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<td>overnight</td>
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<td>30 min.</td>
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DISCUSSION

The hypothesis that a mutation or chromosomal abnormality exists with the IRF-1 gene at 5q31 is not supported by the results because the cell lines each contained the normal two copies of the 5q31 banding region. Instead, the results indicate an unexpected problem, overrepresentation of the 5p arm. The overrepresentation of the 5p arm is not surprising for the non-small cell lung carcinoma cell lines (NSCLC). Comparative Genomic Hybridization (CGH) was conducted on 10 NSCLC cell lines showing frequent gains in the 5p region; unfortunately this CGH study by G. Sonoda is unpublished and the particular cell lines are not known (Testa et al., 1997). CGH is a competitive FISH that uses fluorescent signals to mark normal and tumoral cells; the two signals are then compared by a computer imaging system to indicate regions of DNA amplification or loss (Strachan and Read, 1996). Thus, this study showed that it was quite common for NSCLC cell lines to have amplification of the 5p region. Nevertheless, the overall results of this project show no indication of a microdeletion, in the cytogenetic sense (less than 4 x 10^6 base pairs), in the region 5q31 for any of the cell lines used in this experiment. Other than several 5p amplifications and one reduction (Y-79), there is no indication of a problem with the IRF-1 gene location.

A number of reasons could account for loss of function of the IRF-1 gene region including probe and gene mapping limitations, exon skipping, and problems with in vitro cultures. The main probe that was used was Vysis EGR-1. This probe is reported to hybridize to the 5q31 region. However, it is important to note that the EGR-1 probe does not directly hybridize to the IRF-1 gene location. It hybridizes to the same approximate banding region, 5q31. Thus, the EGR-1 probe would only detect a major problem with this region such as a large deletion, inversion or translocation.

An alternative approach to address this problem would be to construct a "home brew" probe. This is a non-commercial probe that is made by obtaining a sequence of
interest and adding a fluorescent label by standard DNA techniques. If the IRF-1 gene sequence can be obtained, then a "home brew" probe would be another indicator for a genetic abnormality at the microdeletion level. The problem with a "home brew" probe is that the experimenter must run a series of controlled qualification runs to make sure the probe is properly signaling in the target area of interest. With a commercial probe, quality assurance testing is done to show that the probe's efficiency and specificity has been tested. Nonetheless, a "homebrew" probe may prove more beneficial to this experiment.

This experiment must also deal with the limitations of gene mapping. Gene maps can be done by a multitude of different techniques. There are at least 20 different methods of mapping genes (NCBI, 1998). There are also several different groups of people making their own maps. Not all of the gene locations on these maps correspond. In fact, the gene map obtained on IRF-1 (Fig. 16) locates the gene (the red line) on the proximal end closer to the centromere of the 5q 31 region with a large margin of error (the yellow region). The map for EGR-1 (Fig. 17) seems to be located close to the IRF-1 region, but there is no telling how far apart they really are. To further add to the confusion, the gene map of CSF-1R (Fig. 18) shows that this DNA sequence is located in the 5q34 region. However, Vysis maps the CSF probe to the 5q32 region. In the realm of gene mapping there are no clear cut answers.

There is also the possibility of exon skipping. The IRF-1 gene is very susceptible to this problem (Taniguchi, Lamphier, and Tanaka, 1997). Exon skipping is a result of a mutation in either the splice donor or acceptor sequence resulting in the exclusion of either the upstream or downstream exon respectively; the consequent result may be a frameshift, an unstable RNA transcript, or a nonfunctional polypeptide (Strachan and Read, 1996). This phenomenon is commonly the result of a point mutation that is essential in determining the site or termination of RNA splicing (Strachan and Read,
1996). Unfortunately, FISH is not a good indicator of a point mutation. A change in one nucleotide base sequence is not going to affect the hybridization of a probe that is 200 kilobase pairs in length, like EGR-1. Therefore, if exon skipping is occurring in the IRF-1 gene region, the molecular cytogenetic techniques used in this project would not detect any problem.

An automated procedure for fluorescent DNA sequencing of the IRF-1 gene region is a technique that can be applied to address the exon skipping problem. This technique uses primers or dideoxynucleotides with attached fluorophores. Separate fluorescent dyes are used to label the bases and a monitor is used to measure the intensity of fluorescence during electrophoresis. The information is stored electronically, and the technique has proven highly accurate in determining the base sequences (Strachan and Read, 1996). Thus, the non-expressive IRF-1 cell lines used in this experiment could be compared with cell lines expressing IRF-1 using this technique. It may be possible that there is a microdeletion on the nucleic acid level (1-10 base pair deletions) in the non-expressive IRF-1 cell lines. If the deleted bases are at splice donor or acceptor sites, then this would be a good indication of exon skipping.

Another experimental obstacle that exists with this project is the use of tumorgenic in vitro cultures. Dr. Dermer makes the claim that the reason nobody has found a solution to cancer today is because in vitro cultures are so different from in vivo conditions (Spendlove, 1995). Important in vivo conditions such as the existence of three dimensions and extracellular matrix are not found in the laboratory cell cultures. The tumor cell lines that are being studied in the lab have taken on an immortal course of development that may be quite different than the actual cancer found in the body. This may explain why several of the cell lines observed are so abnormal looking. For example, in the small cell lung carcinoma cell line H322 there was an unusual chromosomal breakage. It appears there was a chromatid break in two chromosomes.
resulting in chromatid exchange (Fig. 19). This type of chromosomal configuration is known as a quadriradial (Therman and Susman, 1993). This is not necessarily just an in vitro problem, but it does show that the H322 cell line is capable of developing some genetic abnormalities. This implies that there is some clonal evolution taking place within the cell lines resulting in a diverse population of mutating cells. Growing numerous cultures from single cells and then comparing their genomes could test this clonal evolution hypothesis.

Nevertheless, the results of this study should not be discouraging. The whole chromosome paints (WCP), locus specific probes, and karyotypes confirmed results. For example, looking at the WERI cell line (Figs. 2-5) one can see that the WCP shows the duplicated 5p region indicated by both the EGR-1 and CSF-1R probes. Looking at the WERI karyotype alone it looks like there may be a deletion in one chromosome 5, however the FISH results refute the possibility of this being the 5q31 region. These results were very difficult to obtain. For example, twelve harvests were attempted for the WERI cell line alone. This particular cell line has a lot of residual cytoplasm that impedes the spread of the chromosomes when dropped onto a slide. Therefore, numerous variations in hypotonic solutions and colcemid times were tried (see harvesting attempts). This data could prove to be very beneficial for other researchers new to these cell lines because much time (several weeks) could be saved by looking at the results and choosing the combination treatments that worked best.

In conclusion, the results indicate 5p duplications in the WERI, H1734 and H322 cell lines while the Y-79 cell line showed a deleted 5p region. The IRF-1 gene location on 5q31 appears normal within the limitations of the experiment. Future suggestions of analysis include a “home brew” probe and possibly automated fluorescent DNA sequencing. The “home brew” probe may show a cytogenetic microdeletion in the region because of its higher degree of specificity, and the automated sequencing could test for
exon skipping. The data from this project may prove very valuable for researchers using these particular cell lines or examining a gene in the 5p chromosomal region. The experimentation on IRF-1 can now look toward other technical approaches with the useful knowledge obtained from this project.
Figure 1. EGR-1 probe result for a normal cell line.

Figure 2. EGR-1 probe result for WERI.
Figure 3. CSR-1R probe result for WERI.

Figure 4. WCP #5 probe result for WERI.
Figure 5. Karyotype for WERI
Figure 6. EGR-1 probe result for Y-79.
Figure 7. Karyotype for Y-79
Figure 8. EGR-1 probe result for H1734.

Figure 9. CSF-1R probe result for H1734.
Figure 10. WCP #5 probe result for H1734.
Figure 11. Karyotype for H1734
Figure 12. EGR-1 probe result for H322.

Figure 13. CSF-1R probe result for H322.
Figure 14. WCP #5 probe result for H322.
Figure 15. Karyotype for H322
Figure 16. IRF-1 Gene Map.
**Homo sapiens chromosome 5**

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**Figure 17. EGR-1 Gene Map.**
Homo sapiens chromosome 5

Figure 18. CSF-1R Gene Map.
Figure 19. Abnormal quadriradial for H322.
LITERATURE CITED


Berry, Donna. 1998. Personal Communication. dberry@coml.med.usf.edu

Blanck, G. 1997. IRF-1 Regulation of CIITA. Application for a Research Project Grant from the American Cancer Society Inc.


