Linder, D. and Gartler, S.M. (1965). Glucose-6-phosphate dehydrogenase mosaicism: utilization as a cell marker in the study of leiomyomas. Science 150, 67-69.

Rowley, J.D. (1973). A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by Quinacrine fluorescence and Giemsa staining. Nature 243, 290-293

Background and significance: As discussed in class, somatic mutation as a cause of cancer had lost favor by the 60's. The field theory held that tumors resulted from abnormal growth of a group of cells in response to environmental stimuli. Viruses were another competing idea. Together, these two papers began to shift attention back to somatic mutation.

For the paper by Linder and Gartler, there had been suggestions that tumors arose from a single cell but none were convincing. Some chromosome abnormalities had been shown to be shared by all the cells in a tumor and as Linder and Gartler point out, multiple myeloma cells (a cancer of the immune system cells that make antibodies) had been shown to produce just one of two alleles for the gamma immunoglobulin heavy chain (IgG heavy chain). But other studies showed that different tumor cells could have very different rearrangements and selective overgrowth might also lead to apparent homogeneity. This paper seeks to overcome both these limitations. In earlier work, Gartler had recognized that as a result of X-inactivation in females only one allele of the G6PD locus was expressed in a cell in heterozygous individuals. The two alleles in this case show different mobilities on electrophoresis through starch gels. This type of gel is not used any more, but it would be akin to agarose gels today. Proteins migrate through the gel based on a combination of size and charge and in this case one allele has a charged amino acid in place of a neutral amino acid in the other. One complicating factor for their analysis was that in many tissues groups of adjacent cells all showed the same X-inactivation pattern (patches). Because of this they had to be cautious in their conclusion, but the implication was clear – tumors most likely derived from a single cell.

The paper by Rowley takes the argument to another level. In the early 20th century Boveri had noted mitotic abnormalities in sea urchins led to abnormal development and developed a strikingly modern view of cancer, but even he admitted he had no real knowledge of cancer. But with improved methods for looking at chromosomes in the 50's, scientists looked again at cancer. By 1961 Nowell found a small chromosome – soon dubbed the Philadelphia chromosome – associated with 2 cases of chronic myelogenous leukemia (CML). However, chromosome abnormalities of various kinds were found in other cancer cells, without any consistent patterns. These were dismissed as consequences of abnormal growth, not causes, and the Philadelphia chromosome was ignored. But by 1970, with still further improved cytological methods, Rowley began

examining leukemias again. She first found evidence for a new abnormality in acute myelogenous leukemia – a translocation between 8 and 21 – and then found a second defect in CML patients as described in this paper. These changes occurred early in the course of the disease and were found in many patients for these particular diseases. Eventually, scientists showed that the breakpoints caused the abnormal expression of a gene, leading to abnormal growth and cancer.

Linder and Gartler

Explanation of some terms:
leiomyoma - a tumor of smooth muscle
myometrium – the smooth muscle of the uterus

Suggestions for reading the paper:
The paper is fairly straightforward and the results very clean. The major ambiguity comes from the fact that X-inactivation, while random, occurs fairly early in development. Subsequent growth without mixing results in a patch of cells expressing the same allele. Their first task then is to demonstrate that the patches are relatively small in the uterus.

Questions to answer:
1. Why is only one allele of G6PD detected in clones grown from a single cell?
2. What is the importance of the size of the samples?

Rowley

Explanation of some terms:
transformation – as used here in case 3, a change from a chronic phase to an acute or blast phase, with much more aggressive growth of the leukemic cells.
phytohaemagglutinin – a plant derivative that induces cell division of lymphocytes.
Velban – blocks cells in mitosis

Suggestions for reading the paper:
Don't worry about the details of the methods. The important point is that the combination of quinacrine fluorescence and Giemsa staining combined allowed Rowley to distinguish different human chromosomes and even parts of chromosomes from one another. Don't get bogged down on the chromosome nomenclature. We'll go over that in class. Like many papers that rely on morphological differences, the reproductions often don't show the differences as clearly as the originals, but even in the originals a trained eye is often beneficial. Despite the presence of other abnormalities in some patients, Rowley was not so distracted by these that she lost sight of the common abnormality.

You will see from these first papers that you may have to struggle somewhat with detailed technical aspects of the work, locate library or online resources as necessary to understand the paper, etc. We will attempt to guide you in this to some extent, but part of the value of this course lies in putting yourself in the shoes of these investigators at the time of their work. A scientist has to struggle with unknowns and unproven ideas, and must work within the technical limitations of their time and their experimental system. Have fun!

Questions to answer:

1. Draw the likely event that results in the observed chromosomes. What happens to the ends of 9q and 22q?
2. There are other sources of “dully fluorescing material”. How do they rule them out?