Mapping of the \textit{c-sis} oncogene on human chromosome 22 with respect to the breakpoint associated with chronic myeloid leukaemia

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Chronic myeloid leukaemia (CML) cells are often characterized by the presence of a small chromosome 22, in which most of the q arm has been translocated to chromosome 9. Using cell hybrids containing different parts of chromosome 22 I have mapped the \textit{c-sis} oncogene, which is known to be situated on chromosome 22, to a region distal to the CML breakpoint (22q112) and proximal to 22q13. This demonstrates that \textit{c-sis} is translocated to chromosome 9 in CML cells.

It has become apparent over the past few years that certain chromosomal abnormalities are specifically associated with particular human cancers (1-3). The occurrence of the Philadelphia translocation in patients with chronic myeloid leukaemia (CML) is one of the best-documented examples of such an aberration (3). This involves the translocation of the q11-qter region of chromosome 22 to chromosome 9, resulting in 9q+ and 22q− derivative chromosomes. Furthermore it has now been demonstrated that this is a reciprocal translocation with a small piece of chromosome 9 being translocated to 22 (4).

Recently much interest has been generated by the finding that oncogenes of acute transforming retroviruses have homologues present in normal uninfected cells (5-7). Although a large number of viral oncogenes have been isolated, there are probably fewer than 20 distinct types which have homologues that are present in animal cells. Of particular relevance to CML have been the observations that the cellular homologue (\textit{c-abl}) of the transforming sequence of the Abelson murine leukaemia virus is located on chromosome 9 (8,9), and that the cellular homologue (\textit{c-sis}) of the Simian sarcoma virus oncogene is located on chromosome 22 (10).

It has been postulated that one mechanism by which cancers might arise is the activation of a cell oncogene due to its translocation to a region which is under the influence of a regulatory DNA sequence (11,12). \textit{c-abl} has now been shown to be translocated from chromosome 9 to the 22q− derivative in CML (4). It is therefore of great interest to determine how close the \textit{c-sis} oncogene is located to the CML-specific breakpoint, and if it is translocated to the 9q+ chromosome.

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Materials and Methods

Cells and cell culture

The somatic-cell hybrids were provided by Dr. A. Guerts van Kessel, and have been fully described elsewhere (13-15). 1/22AM and 33-11 hybrids were derived from fusion of human leucocytes (carrying translocations 1:22 and X:22) with Chinese-hamster a3 and E36 cells respectively. 14CB hybrids were derived from fusion of human CML cells with hamster E36 cells. The PgMo-22 hybrid was derived from fusion of human CML cells (from a different patient to those used for the 14CB hybrids) with mouse Pg-19 cells.

The cells were cultured in Hams SF-12 medium supplemented with 10% foetal calf serum and grown at 37°C in an atmosphere of 5:95% CO2:air.

Hybridization analysis

High-molecular-weight DNA was prepared from the cell lines and cell hybrids (16). Each of the DNA samples was restricted with Bam H1, size-fractionated on a 1% agarose gel, and transferred to nitrocellulose filters (17). The c-sis probe, pL 335, was provided by Dr. R. C. Gallo (17). This was nick-translated (19) to produce a probe with a specific activity of 8 x 10^7 c.p.m./μg. After hybridization the filters were subjected to two 30-min washes in 2 x SSC, and two 30-min washes in 0.1 x 5SC, 0.1% SDS at 65°C.

Results and Discussion

Fig. 1 shows the hybridization of a human c-sis oncogene probe to DNA samples prepared from a number of cell lines and cell hybrids. The probe hybridized strongly to a 1.7-kb Bam H1 fragment of the human DNA control, which is the characteristic hybridization pattern observed with all of the human samples I have examined (unpublished observations). Hybridization was also observed with a partial digest fragment at 7 kb. All of the hamster CHO and CHO x human hybrid DNA samples showed a strong hybridization to the c-sis probe at approx. 20 kb. This represents hybridization to the hamster c-sis oncogene. However, only a few of the hybrids, and none of the parental CHO cell lines, exhibited hybridization to the human specific 1.7-kb fragment. In particular the human 1.7-kb band was obvious in the 14CB-21A hybrid (which contains the 9q+ chromosome from a CML patient) but was absent from the 14CB-5A lane (which contains the reciprocal 22q- Philadelphia chromosome from the same patient).

The hybridization of the c-sis probe to the mouse x human hybrid (PgMo-22) DNA is also shown in Fig. 1. This hybrid contains a 9q+ chromosome but from CML cells of a different patient to those used to construct the 14CB-21A hybrid. Here again hybridization to a 1.7-kb fragment was observed. Hybridization was also observed with the parental mouse c-sis fragment at 1.5 kb. The hybridization of the c-sis probe to the 9q+ -containing hybrids showed only a faint 1.7-kb band because there was a low representation of the human 9q+ chromosome in these cells.
Fig. 1. Mapping of c-sis on human chromosome 22. DNA from a range of cells, containing different parts of chromosome 22, was restricted with Bam H1, fractionated on a 1% agarose gel, and transferred to a nitrocellulose filter. The resulting filter was then hybridized with a nick-translated c-sis clone, pL335.

The different translocated parts of human chromosome 22 present in each of the cell hybrids are summarized in Table I. It is clear from the data presented in this paper that c-sis is localized in the region q112-q13 on chromosome 22, and is translocated to chromosome 9 in CML cells. Although this is a more precise localization than previously described (10) it is still not clear how closely c-sis is linked to any putative chromosome 9 activating sequence on the 9q+ derivative.

The c-abl situation appears to be better defined, as this cell oncogene is known to be translocated to the 22q− derivative (4) and must be close to the breakpoint. Recently I have mapped the lambda light-chain constant region (C\(\lambda\)) immunoglobulin locus on chromosome 22 to just proximal to the CML breakpoint (MH Goyens, BD Young, A Geurts van Kessel, A de Klein, G Grosveld, CR Bartram & D Bootsma,
Table 1. Summary of the human chromosome-22 regions present in each of the cell types used in Fig. 1

(+) represents hybridization of the c-sis clone to the human 1.7-kb fragment.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Part of human chromosome 22 present</th>
<th>Hybridization of c-sis probe to DNA</th>
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<tbody>
<tr>
<td>Human peripheral blood</td>
<td>22</td>
<td>+</td>
</tr>
<tr>
<td>CH0 (a3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AM-21</td>
<td>22q13 + qter</td>
<td>-</td>
</tr>
<tr>
<td>AM-27</td>
<td>22pter + q13</td>
<td>+</td>
</tr>
<tr>
<td>CH0 (E36)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>33-11</td>
<td>22pter + q11 and 22q11 + qter</td>
<td>+</td>
</tr>
<tr>
<td>33-11-6TG</td>
<td>22q11 + qter</td>
<td>+</td>
</tr>
<tr>
<td>14CB-5A</td>
<td>22pter + q112</td>
<td>-</td>
</tr>
<tr>
<td>14CB-21A</td>
<td>22q112 + qter</td>
<td>+</td>
</tr>
<tr>
<td>PgMo-22</td>
<td>22q112 + qter</td>
<td>+</td>
</tr>
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manuscript submitted for publication). Thus c-abl and this immunoglobulin locus may be closely linked, possibly bringing c-abl under the influence of an immunoglobulin enhancer sequence (20). Evidence in support of such a close linkage has been provided by a study of the CML cell line, K562, in which both c-abl and C3 genes were found to be amplified (21). However, c-abl does not appear to be rearranged after translocation (4) and does not exhibit measurable increases in transcription in leukaemia cells (21). Furthermore, it is the 22q- derivative which is characteristic of CML, and although in most patients the observed abnormality is a 9:22 translocation, chromosomes other than chromosome 9 can be involved. It is therefore still unclear which of these oncogenes, if either, has a role in establishing a leukaemic condition in myeloid cells.

Acknowledgements

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References