

# Lack of structural rearrangement in *c-kit* and stem cell factor genes in Hong Kong Chinese patients with myelodysplastic syndromes or acute myeloid leukaemia

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Stem cell factor is a haemopoietic growth factor that interacts with the *c-kit*-encoded transmembrane tyrosine kinase receptor during signal transduction in haemopoietic progenitor stem cells. We have screened 127 Chinese patients with myelodysplastic syndromes or acute myeloid leukaemia for structural rearrangements in the stem cell factor and *c-kit* genes using Southern blot analysis. No structural rearrangements were detected in any of the bone marrow samples that were tested. It seems that structural rearrangements in the stem cell factor and *c-kit* genes are rare in Hong Kong patients who have a haematological malignancy.

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## Introduction

Stem cell factor (SCF) is a haemopoietic growth factor whose receptor is a transmembrane protein tyrosine kinase (PTK) that is encoded by the *c-kit* proto-oncogene. In the mouse genome, SCF and its receptor are encoded at the steel (*Sl*)<sup>1</sup> and white spotting (*W*)<sup>2</sup> loci respectively. Deletion or loss-of-function mutations of the murine *SCF* and *c-kit* genes result in pleiotropic developmental defects which include macrocytic anaemia, mast cell deficiencies, and white spotting of the skin.<sup>3,4</sup>

Gain-of-function mutations in *c-kit* and other genes of the receptor PTK family have been described in various haematological malignancies.<sup>5,6</sup> A point mutation in the extracellular domain of the receptor PTK for human colony-stimulating factor 1 (encoded by the *c-fms* proto-oncogene) has been identified in some patients with myelomonocytic leukaemia.<sup>5</sup> A

point mutation in the catalytic domain of *c-kit* has also been identified in peripheral blood mononuclear cells of patients with mastocytosis<sup>7</sup> or mast cell leukaemia,<sup>8</sup> and substitutes valine for aspartic acid at codon 816. In vitro experiments have shown that this point mutation causes ligand-independent activation of the *c-kit*-encoded receptor PTK and the induction of gene expression that is qualitatively and quantitatively different from that following the activation of wild-type *c-kit*-encoded receptor by SCF.<sup>8</sup> Mutations that cause constitutive activation of *c-kit* can also confer factor-independent growth and tumorigenicity in factor-dependent haemopoietic cell lines.<sup>6</sup>

Studies of patients with acute myeloid leukaemia (AML) from North America<sup>9</sup> and Japan<sup>10</sup> have shown that *c-kit* mRNA and protein product are expressed in the blast cells in >80% of patients. Structural alterations of the *c-kit* gene have also been reported in some AML patients.<sup>11</sup> It has been suggested that *c-kit* is involved in myelodysplastic syndromes (MDS).<sup>12,13</sup> These syndromes are characterised by pancytopenia, hypercellular bone marrow, and a high incidence of transformation to AML. Both in vivo and in vitro haemopoiesis are generally impaired in MDS.<sup>12</sup> Backx et al<sup>12</sup> observed that erythroid colony formation in response to erythropoietin was reduced in bone marrow cells from patients with MDS. The addition of SCF improved in vitro erythropoiesis in 'low-risk' subtypes of MDS such as refractory anaemia and refractory

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anaemia with ringed sideroblasts, but not in refractory anaemia with excess blasts, refractory anaemia with excess blasts in transformation, and chronic myelomonocytic leukaemia.<sup>12</sup> The lack of responsiveness to SCF in the latter group of MDS is suggestive of qualitative or quantitative defects of the *c-kit*-encoded receptor. Similar findings have also been reported by Verbeek et al.<sup>13</sup> These data suggest that the SCF/Kit pathway may play an important role in leukaemogenesis.

We have previously found that approximately 75% of bone marrow samples from a group of AML patients in Hong Kong did not express *c-kit* mRNA or protein in the bone marrow.<sup>14</sup> In this study, we analysed the structure of the *c-kit* gene in bone marrow from the same AML patients to test whether structural rearrangements in this gene are responsible for its lowered expression. We also tested bone marrow from some Hong Kong MDS patients, and tested for structural rearrangements in the *SCF* gene.

## Materials and methods

### Samples

Bone marrow samples were taken from 51 Hong Kong patients with AML, 4 with acute lymphoid leukaemia, 15 with chronic myelogenous leukaemia (CML) in the chronic phase, 15 with CML in the accelerated phase or blastic crisis, 4 with essential thrombocythaemia, 5 with polycythaemia rubra vera, 8 with myeloproliferative disorder, 4 with thrombocytosis, 15 with MDS, 6 with lymphoma, and from 10 healthy bone marrow donors. Patients were diagnosed as having AML<sup>15</sup> or MDS<sup>16</sup> according to the French-American-British classification system. All CML patients were Philadelphia chromosome-positive; blastic transformation was defined as the presence of >30% blasts in the blood marrow sample. Cytochemistry and immunophenotyping studies were carried out on the blast cell population.

### Southern blot analysis

High molecular weight DNA was obtained from bone marrow samples by proteinase K digestion, followed by sodium dodecylsulphate and phenol/chloroform extraction. DNA samples (2.5-10.0 µg each) were digested with the restriction endonucleases *EcoRI*, *BglII*, *BamHI*, or *HindIII*. The digested DNA fragments were separated according to size on a 0.8% (w/v) agarose gel. Southern blot analysis and hybridisation were performed as previously described.<sup>11</sup> The hybridisation probes used were a 1.25 kb *SstI* fragment of human *c-kit* cDNA (Dr A Ullrich, American Type Culture Collection, Rockville, Maryland, USA) that

corresponded to the 5' untranslated region and part of the extracellular domain of the *c-kit* gene; and a 431 bp *SmaI-SphI* fragment of human *SCF* cDNA (Dr KM Zsebo<sup>1</sup>) that corresponded to part of exon III through part of exon VII of the *SCF* gene. The probes were radioactively labelled with [ $\alpha$ -<sup>32</sup>P]-dCTP to a specific activity of >1x10<sup>8</sup> dpm/µg using an oligonucleotide labelling kit (Oligolabelling kit, Pharmacia Biotech, Uppsala, Sweden).

## Results

All lanes from all patients, irrespective of the type of haematological disorder, expression of *c-kit* mRNA or protein, or restriction endonuclease used in the DNA digestion, gave identical bands in the Southern blot to the wild-type lanes. Thus, the *SCF* and *c-kit* cDNA probes used in this study failed to detect any structural rearrangements or gross deletions in the *SCF* and *c-kit* genes from the bone marrow DNA samples from Hong Kong patients with MDS or AML. Representative results are shown in the Figure.

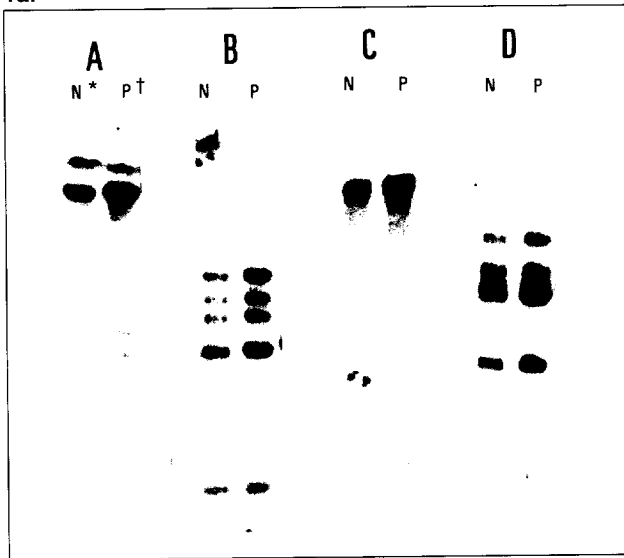
## Discussion

We extracted the bone marrow DNA from Hong Kong patients with a variety of haematological disorders and probed it with cDNA from *c-kit* and *SCF* genes. We did not detect any structural abnormalities in these genes.

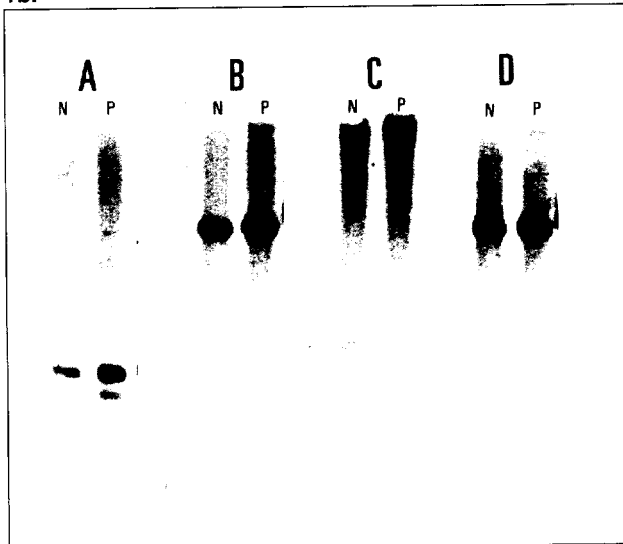
Of the 127 patients, 51 (40%) had AML and of these, 13 (25%) expressed the *c-kit* mRNA or protein product.<sup>14</sup> This contrasts with the high frequency of expression observed in Caucasian and Japanese AML patients.<sup>9,10</sup> This study shows that structural rearrangements or gross deletions in the *c-kit* gene are not the cause of the lack of its expression in the majority (75%) of patients tested. We used the cDNA corresponding to part of the extracellular domain of the *c-kit* gene as the probe, rather than using cDNA from the catalytic domain, which bears sequence homology to other genes of the receptor PTK family. The probe used in this study is similar to that used by the research groups in Japan and Canada to investigate *c-kit* mRNA expression<sup>9,10</sup> and to show that the *c-kit* gene was also not rearranged.<sup>9</sup>

It has been suggested that leukaemic transformation occurs in a *c-kit*-positive stem cell.<sup>9,10</sup> We have shown that this is not the case in 75% of patients,<sup>14</sup> which may indicate a differential pathogenesis of AML in Hong Kong. The present study shows that gross deletion and rearrangement in the *c-kit* and *SCF*

1a.



1b.



\*N bone marrow DNA from healthy donor  
 †P patient with haematological malignancy

#### Fig. Southern blot analysis of bone marrow DNA

Blots were probed with radioactively labelled (1a) *c-kit* and (1b) *SCF* cDNA probes; bone marrow DNA was digested with the following restriction endonucleases: (A) *EcoRI*, (B) *BglI*, (C) *BamHI* and (D) *HindIII*

genes do not play a role in leukaemic transformation. The results suggest that a pathway independent of the products of these genes is involved. An SCF/Kit-independent pathway has been described in some haemopoietic cell lines; Ikuta et al<sup>17</sup> and Sutherland et al<sup>18</sup> have demonstrated that haemopoiesis can be maintained with different non-synergising factors that do not include SCF. The protein encoded by the *FLT3* gene, another member of the receptor PTK family may be involved in leukaemic transformation.<sup>19</sup> High levels of *FLT3* expression have been detected in approximately 92% of AML cases in Italy,<sup>20</sup> with subtypes ranging from M0 to M7, and in CML patients in

myeloid blastic crisis. A study of *FLT3* gene expression in Hong Kong AML patients may thus be of interest.

Our findings may have implications in terms of the treatment and prognosis of Hong Kong patients. The expression of *c-kit* has been reported to be an independent prognostic factor.<sup>21</sup> Ashman et al<sup>21</sup> studied 71 patients with newly diagnosed acute non-lymphoblastic leukaemia by using the monoclonal anti-Kit antibody YB5.B8. Patients whose blast cells were YB5.B8-positive had a lower remission rate and poorer overall survival rate than those whose blast cells failed to bind the antibody. If the SCF/Kit pathway were not involved in Hong Kong patients, then *c-kit* analysis might not always give additional prognostic value. Also, therapy directed at the SCF/Kit pathway (eg *c-kit* antisense oligonucleotides) would be of limited benefit.

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