V. Summary

In this work, a female patient with a phenotype resembling Costello syndrome and carrying an apparently balanced *de novo* translocation t(1;22)(q24.3;q13.1) was studied. In general, balanced chromosomal rearrangements associated with a Mendelian disorder are powerful tools for mapping novel disease genes. Molecular analysis of the breakpoint regions may help to identify interrupted gene(s) responsible for the phenotype of the patient. By mutation analysis of gene(s) located in the breakpoint regions in patients with the same disease phenotype and a normal karyotype, the disease causing gene can be identified by discovering additional mutations. Initially, a breakpoint spanning PAC clone for the breakpoint on 1q24.3 as well as a cosmid clone overlapping the breakpoint on chromosome 22 were identified by fluorescence *in situ* hybridization. Subsequent database analysis of the DNA sequence revealed that the gene encoding the platelet-derived growth factor beta *(PDGFB)* is located on the insert of the cosmid. Characterization of the breakpoint regions at the molecular level showed that the translocation patient carries a mosaic of two derivative chromosomes 1 in her peripheral blood lymphocytes, in one of which the coding region of the *PDGFB* gene was disrupted. Both the initial translocation and the secondary intrachromosomal rearrangement appear to have occurred by non-homologous (illegitimate) recombination.

In the 1q24.3 breakpoint region of the patient with the 1;22 translocation and a phenotype resembling Costello syndrome, a putative novel gene was identified and partially characterized suggesting that it represents a pseudogene. The *PDGFB* gene was found to be disrupted by the breakpoint in 22q13.1. The considered pattern of inheritance for Costello syndrome is autosomal dominant and thus, disruption of one *PDGFB* allele by the translocation could result in the Costello phenotype in this patient. Therefore, the *PDGFB* gene was a good candidate gene for Costello syndrome. Mutation analysis of *PDGFB* and five genes belonging to the *PDGF/R* family (*PDGFA*, *PDGFC*, *PDGFD*, *PDGFRA*, and *PDGFRB*) revealed no pathogenic mutations in 18 sporadic patients with Costello syndrome. These negative results prompted us to re-evaluate the clinical symptoms of the translocation patient. Although some of the typical clinical features of patients with Costello syndrome were also found in the translocation patient, the majority of them was absent. Therefore, it seems likely that the translocation patient shows a ‘unique’ phenotype not resembling any known and well-defined syndrome.

In total RNA isolated from lymphocytes of the translocation patient, four fusion transcripts consisting of *PDGFB* exons and various DNA fragments located in the breakpoint region on

1q24.3 were identified. In two of the mRNAs, exon 6 of *PDGFB*, encoding the 41 C-terminal amino acid residues, was absent. *PDGFB* exon 6 encodes a stretch of basic amino acids, the retention motif, that mediates interaction of PDGFB with components of the extracellular matrix. After maturation and cleavage of the C-terminal retention sequence, the PDGFB molecule becomes diffusible. Immunofluorescence analysis showed that the fusion protein between PDGFB wild-type and the green fluorescence protein EGFP was dispersed and formed a network-like structure in the extracellular matrix whereas the two aberrant PDGFB-EGFP fusion proteins (without retention motif) were localized in aggregates. We speculate that the biological consequences of the mutant *PDGFB* allele might have contributed to the disease phenotype of the translocation patient.

In an alternative attempt to identify the disease gene for Costello syndrome, we searched for functional candidate genes for this disorder. Mutation analysis of the *FOXO1A*, *TGFB1*, and *LMNA* genes was performed and no pathogenic mutation was identified in sporadic patients with Costello syndrome. Nevertheless, the identification of disease genes for syndromes showing phenotypic overlap with patients with Costello syndrome, like Noonan syndrome, may help to select novel functional candidate genes for Costello syndrome. For example, *PTPN11*, one causative gene for Noonan syndrome, encodes the protein tyrosine phosphatase SHP2 implicated in epidermal growth factor (EGF) dependent signaling pathways. These data together with the phenotypic features observed in various mutant mice suggest that the gene encoding the epidermal growth factor receptor (*EGFR*) is a promising candidate gene for Costello syndrome.