Summary
Cohesinopathies are human developmental disorders caused by inherited defects in cellular components controlling the process of sister chromatid cohesion. This cohesion mechanism takes care of keeping the sister chromatids close together from the stage of DNA replication up until mitosis. Central player in this process is the cohesin complex, which is regulated by several other proteins. In order for the cohesin complex to become cohesive, sister chromatid cohesion is established at the replication fork by a protein that is called Eco1 in yeast. There are two Eco1 orthologs in humans: ESCO1 and ESCO2. Mutations that inactivate ESCO2 are responsible for Roberts syndrome (RBS), one of the two cohesinopathies described so far. The other cohesinopathy, Cornelia de Lange syndrome (CdLS), is caused by mutations in genes encoding either cohesin subunit SMC1 or SMC3, or in the cohesin loader NIPBL. Sister chromatid cohesion has been implicated in DNA repair and prevention of tumorigenesis, because mutations that interfere with this process lead to increased sensitivity to DNA-damaging agents, while such mutations have also been detected in a proportion of carcinomas.

In this thesis, our knowledge about sister chromatid cohesion in human development and carcinogenesis was further explored. After a general introduction in Chapter 1, we introduce a new syndrome, Warsaw breakage syndrome (WABS), in Chapter 2. This syndrome, caused by mutations in the cohesion-related helicase DDX11, can now be considered as the third cohesinopathy, next to RBS and CdLS. The fact that cells from the WABS patient, like RBS cells, are hypersensitive to the DNA cross-linking agent mitomycin C, might lead to a diagnostic overlap between RBS, WABS and the chromosomal instability syndrome Fanconi anemia, which also features such hypersensitivity. This clinical and diagnostic overlap was discussed in Chapter 3. A clear distinction between these different diseases could be made when metaphase chromosomes were carefully examined for cohesion defects.

To further explore the role of ESCO2 in the process of sister chromatid cohesion and DNA repair, a functional characterization of the protein was initiated by ectopic ESCO2 expression in RBS cells (Chapter 4). By comparing RBS cells with cells that were functionally complemented with epitope-tagged ESCO2, it was shown that ESCO2 is involved in the defence against DNA-damaging agents that specifically act in S phase. In line with this, a relatively high ESCO2 expression was observed in S phase, which was regulated by the proteasome. No defects were found in homologous recombination, as determined by the capacity of the cells to form Rad51 foci and to engage in the formation of sister chromatid exchanges.

In Chapter 5, the ESCO2 counterpart ESCO1 was studied by expression of mCherry-tagged ESCO1. A clear distinction between the properties of ESCO1 and ESCO2 was found: while ESCO2 expression was cell cycle regulated, ESCO1 was present throughout the cell cycle, while its expression was independent of the proteasome. In addition, a difference in
localization was observed: a nuclear localization with accumulation in the nucleoli was found for ESCO2, whereas ESCO1 had a more diffuse distribution throughout the nucleus. During cell division, ESCO1 clearly associated with chromosomes in their condensed stage, which was not observed for ESCO2. Interestingly, whereas ESCO1 and ESCO2 have been claimed to be functionally non-redundant, overexpressed mCherry-tagged ESCO1 was able to partially correct for the cohesion defects found in RBS cells, suggesting at least partial redundancy in their functions.

Because of its role in human development, and because indications existed indicating an involvement of sister chromatid cohesion in the prevention of carcinogenesis, we examined the possible association of defects in sister chromatid cohesion with the cancerous state (Chapter 6). In two out of twelve colon carcinoma cell lines, cohesion defects were found. In head and neck squamous cell carcinoma cell lines we found an even higher percentage of cell lines displaying cohesion defects (35%). In one of these cell lines, mutations were found in the PDS5A gene, leading to loss of this cohesin-associated protein. In addition, increased levels of a CHTF18 splice-variant led to reduced protein levels in two cell lines (independently isolated from the same patient), both of which exhibited cohesion defects. These data suggest that there might be a role for defects in sister chromatid cohesion in the development of cancer.

In Chapter 7 the results of the studies described in this thesis are discussed and future perspectives and possible applications are proposed. In conclusion, this thesis describes the significance of sister chromatid cohesion in human development and possibly carcinogenesis. It provides new diagnostic insights in the cohesinopathies, and brings us closer to a better understanding of the cohesion process in the human cell.