Chapter 8

Summary and conclusion

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Conclusion

Future perspectives

Summary

Chapter 1 consists of a review of the present knowledge of the clinical characteristics, the genetics, heredity and tumor biology of paragangliomas and pheochromocytomas.

Chapter 2 is a review of the literature on recent advances in the understanding of the genetics of paragangliomas. Current insights as well as future directions are discussed, showing that major progress has been made in this field since the discovery of mutations in SDH genes as a cause of paraganglioma syndrome.

Chapter 3 presents an overview of the relative frequency of mutations in SDH genes that are associated with paraganglioma-pheochromocytoma syndrome in the Netherlands. In this study, we find that the large majority of mutations in SDH subunits or co-factors involve SDHD, followed by SDHAF2 and SDHB, whereas SDHC mutations are extremely rare. In addition, we found that the overwhelming majority of SDH-mutation carriers in the Netherlands carry one of only 6 Dutch founder mutations in SDHAF2, SDHB and SDHD. Out of these 6 founder mutations, the p.Asp92Tyr founder mutation in SDHD is by far the most prevalent, accounting for 69% of all Dutch SDH mutation carriers. Both the dominance of SDHD founder mutations and the limited genetic heterogeneity among SDH mutation carriers are unique to the Netherlands.

Chapter 4 consists of a study of the mutation status and clinical characteristics of a series of 236 Dutch head and neck paraganglioma patients treated at the Leiden University Medical Center. In line with the findings in chapter 3, this Dutch patient series is characterized by a high prevalence of *SDHD* mutations. Contrasting with studies performed in other European countries, the majority (80%) of the patients in this cohort present with a family history positive for paraganglioma syndrome. Surprisingly, we find that even in patients with a negative family history for paragangliomas, hereditary forms of the paraganglioma syndrome are found in the majority of cases. In this patient group too, the disease is frequently linked to mutations in the *SDHD* gene.

The clinical consequences of SDHD mutations are also evaluated in this chapter: an early mean age at onset of paraganglioma syndrome of 38 years, a high risk of multiple paragangliomas (73%), a risk of concurrent pheochromocytomas (13%) and extra-adrenal paragangliomas (8%), and a small risk of metastatic disease (2%). Carriers of mutations in SDHAF2, SDHB and SDHC are also identified in this patient series, as well as patients without a mutation in any of these genes, but these subgroups constitute a small minority of the Dutch head and neck paraganglioma population. We argue that the high prevalence

of Dutch SDHD founder mutations, as well as the small numbers of SDHB-linked and SDH mutation-negative cases imply that the prevalence of paraganglioma syndrome may be higher in the Netherlands than elsewhere.

In **chapter 5**, a large, multigenerational Dutch paraganglioma family linked to the D92Y (also p.Asp92Tyr or c.274G>T) founder mutation in SDHD is described. The SDHD.D92Y mutation is the dominant cause of head and neck paragangliomas in the Netherlands. As all mutation carriers in this family carry the same mutation, we were able to describe its phenotype in detail, and found that it does not differ much from the phenotypes of other *SDHD* mutations. In addition, by including a large number of asymptomatic family members, we were able to make accurate calculations of the penetrance of this founder mutation, both for the occurrence of paragangliomas as well as for symptomatic disease. We found, in accordance with our expectations, no maternal transmission of SDHD-linked disease. We found that a paternally transmitted mutation confers a high lifetime risk of paragangliomas of 87%, however this is lower than previous estimates. Moreover, we found that the life time risk of developing paraganglioma-associated symptoms is considerably lower (57%).

Chapter 6 comprises of a gene expression study, comparing the expression levels of more than 8.000 genes in SDHD-linked, PGL2-linked and sporadic paragangliomas. At the time of analysis, the exact identity of the *PGL2* gene was unknown, and an attempt was made to define its function and thus clarify its identity on the basis of a distinctive gene expression profile. However, no significant differences could be identified in the gene expression of these genetic subgroups. Even in selected subsets of genes that are known or suspected to play a role in the pathways that lead to paraganglioma tumorigenesis, no differences could be found. We therefore hypothesized that this might be because *SDHD* and *PGL2* mutations exert a similar effect on the functionality of succinate dehydrogenase. We now know, since the identification of *SDHAF2* as the *PGL2* gene and the discovery of its role in SDH activity, that this is indeed the case.

In **chapter 7**, a model is put forward to explain the peculiar inheritance pattern in SDHD-linked paragangliomas and pheochromocytomas. It has been known for some time that tumors almost never occur in *SDHD* mutation carriers that have inherited the mutation via their mother. However, if the same mutation is inherited via the father, the risk of developing paraganglioma syndrome is very high. This mode of inheritance causes paraganglioma syndrome to skip generations and is consistent with maternal imprinting of the *SDHD* gene. However, methylation or imprinting of the SDHD gene itself has never been established, and bi-allelic expression of *SDHD* has been demonstrated in non-

paraganglion tissues. In addition, SDHD acts as a tumor suppressor gene in paraganglioma syndrome, i.e. the loss of the wild-type SDHD allele is a prerequisite for paraganglioma development, which would be counter-intuitive if the wild type allele was already silenced by methylation.

In this study, we observe that in SDHD-linked paragangliomas and pheochromocytomas, the LOH does not only target the wild-type *SDHD* allele, but involves the whole maternal chromosome 11, suggesting that the loss of another gene on chromosome 11 is essential for paraganglioma development. As this somatic loss consistently affects the maternal chromosome 11 copy, it is likely that this other gene is exclusively maternally expressed, thus paternally imprinted. These conjectures all point in the direction of genes located within the 11p15.5 region, a major imprinted gene cluster in the human genome, and we hypothesize that a paternally imprinted gene on 11p15.5 acts as an additional tumor suppressor in SDHD-linked paraganglioma syndrome.

According to this model, loss of the wild-type *SDHD* allele alone is insufficient for tumor formation in *SDHD* mutation carriers. Only upon loss of both the wild-type *SDHD* allele on 11q23 and the active maternal copy of a tumor suppressor gene on 11p15.5, tumor formation will occur. In paternally, but not in maternally derived *SDHD* mutation carriers, this can be achieved by a single event: non-disjunctional loss of the maternal chromosome 11 (chapter 1, figure 6). The virtually exclusive paternal transmission of the disease can be thus explained by a somatic mechanism targeting both the wild type *SDHD* gene on 11q23 and the maternal copy of a paternally imprinted gene on 11p15.5, rather than imprinting of SDHD itself. This model could explain the parent-of-origin-dependent inheritance in SDHAF2-linked paraganglioma as well, as SDHAF2 is also located on the long arm of chromosome 11. It furthermore leaves room for maternal inheritance of disease, as other mechanisms inactivating both the wild type *SDHD* allele and the maternal 11p15.5 region could also cause tumor formation, however, maternal transmission is predicted to occur very rarely as this would require more complex somatic rearrangements.

Conclusion

Since the discovery of mutations in SDH genes as the cause of hereditary head and neck paragangliomas in the year 2000, great progress has been made in the identification of pathogenic mutations, the description of phenotypic differences in between the causative genes, and the understanding of the molecular biology linking SDH defects with neoplastic growth.

In this thesis, the relative importance of the pathogenic mutations in the SDH genes in the Netherlands is elucidated, revealing a remarkable role of founder effects, especially in *SDHD*, but also in *SDHB* and *SDHAF2*. The prevalence of Dutch founder mutations has been recognized before, but their absolute dominance, especially of the SDHD.D92Y mutation, and the relative low numbers of other SDH mutations in the Dutch population represent a new insight. We argue that these findings may underlie an increased prevalence of head and neck paragangliomas in the Netherlands.

A comprehensive understanding of the natural course of the disease and the risk of developing multifocal, adrenal, metastatic, or symptomatic disease is important in the clinical decision making in head and neck paraganglioma patients. As complete eradication of paragangliomas is not always possible or may confer a high risk of morbidity, especially in bilateral disease, the consequences of any treatment must always be weighed against the consequences of no intervention. By studying a large patient cohort and an extended paraganglioma family, we were able to characterize SDHD-linked paraganglioma patients, and thus the majority of the Dutch head and neck paraganglioma population, by an early mean age at diagnosis (26.5-37.9 years), a high rate of multiple tumors (65-74%), an intermediate risk of concurrent pheochromocytomas (8-21%), and a low risk of malignancy (2-3%). In addition, we found that whereas mutations in SDHD confer a high lifetime risk of developing a paraganglioma, not all paraganglioma patients develop tumor-related symptoms. Therefore, bearing in mind the words of Le Compte ("*the greatest danger to these patients is the treatment rather than the disease"*), a conservative treatment strategy seems appropriate in the majority of Dutch head and neck paraganglioma patients.

As the Dutch SDHD-linked phenotype does not differ significantly from the SDHD-linked phenotype found elsewhere in Europe or the United States, we furthermore conclude that the high prevalence of Dutch founder mutations in SDHD is a reflection of specific aspects of the Dutch demography and socio-economic history, rather than a result of environmental factors such as residential altitude.

Another important feature of SDHD-linked paragangliomas is the virtually absent maternal transmission of the disease. We have shown that the 'second hit' in SDHD-linked paragangliomas involves not only the wild type SDHD allele but the whole maternal chromosome 11 copy, suggesting a model that involves the combined loss of the wild type *SDHD* allele and a maternally expressed, paternally imprinted tumor suppressor located on 11p15.5 as an essential step in SDHD-linked paraganglioma formation. The almost exclusive paternal transmission of disease would then be the result of the colocation of this imprinted tumor suppressor and the wild type *SDHD* allele on the maternal copy

of chromosome 11. As of yet, the paraganglioma tumor suppressor on 11p15.5 has not been identified with certainty, but if substantiated, this model explains the parent-of-origin dependent inheritance in the absence of imprinting of the *SDHD* gene itself. The fact that exclusive paternal inheritance of disease is also found in SDHAF2-linked paraganglioma families supports this model, as *SDHAF2*, like *SDHD*, is located on the long arm of chromosome 11 (11q13).

The proposed model furthermore explains the observation that true maternal transmission of SDHD-linked disease is possible, but rare. Simultaneous loss of the wild type *SDHD* allele and the active tumor suppressor allele can be achieved in a single event in case of a paternally inherited *SDHD* mutation (by loss of the whole maternal chromosome 11 copy), whereas it would require at least 2 separate hits targeting separate regions and/or separate copies of chromosome 11 in case of a maternally inherited mutation, a sequence of events that is almost certainly less likely to occur in vivo. In support of this model, additional events targeting the maternal 11p15.5 region have indeed been identified in the recently reported rare occurrences of true maternal inheritance of SDHD-linked disease.

The model could also explain the higher penetrance of SDHAF2 and SDHD-linked disease as opposed to SDHB- and SDHC-linked disease. As explained above, SDHD- and SDHAF2-linked tumorigenesis may be initiated by a single event targeting the whole maternal chromosome 11 copy. Assuming that loss of the maternal tumor suppressor allele on 11p15.5 is a prerequisite for the development of all SDH-linked paragangliomas, paraganglioma formation would require at least 2 separate hits, targeting the maternal 11p15.5 region and the *SDHB* or *SDHC* wild type allele on chromosome 1 in SDHB- and SDHC-linked disease.

In broader terms, the model for the parent-of-origin-dependent inheritance of SDHD-linked paragangliomas illustrates the importance of the location of disease genes on the genome, and demonstrates that even in alleged monogenetic diseases, multiple genes may be involved as essential initiators of disease or modifiers of disease risk.

Future perspectives

In order to further clarify the problem of the parent-of-origin-dependent inheritance in SDHD- and SDHAF2-linked paragangliomas, future research is needed into the role of the 11p15.5 region both in SDHD- and SDHAF2-linked paragangliomas as well as in SDHB-, SDHC- and VHL-linked cases. The identification of the additional tumor suppressor gene or genes responsible for this phenomenon will almost certainly shed more light on the molecular mechanisms that underlie paraganglioma formation and probably help explain aspects of tumor behavior. In general, it will broaden our understanding of the significance of modifier genes for the occurrence and form of disease.

Paraganglioma research has improved our insight into the link between hypoxia regulation, metabolic disruptions and tumor formation. However, in spite of the progress made, some tantalizing questions still remain unanswered. It is presently unknown why germ line mutations in genes encoding SDH, a complex that is so vital to the energy supply of cells, preferably produce tumors in the paraganglion system, and do not (with the exception of SDHA mutations) cause a more generalized or severe disease phenotype. It is furthermore surprising that mutations in different subunits of the same complex (SDH), all resulting in SDH deficiency, give rise to quite distinct paraganglioma syndromes. On the other hand, it is equally surprising that mutations in genes with such different functions as the SDH genes and *TMEM127* or *MAX*, all cause the same tumor type. These unresolved issues illustrate the long way to go before the different, interacting molecular mechanisms that cause paraganglioma are unraveled. As hypoxia pathway signaling and the switch to aerobic glycolysis are characteristics of a large variety of neoplasms, elucidating these pathways may have ramifications beyond the field of paragangliomas and pheochromocytomas. Already, several agents have been identified that exert a possible anti-cancer effect through interaction with key components of the hypoxia pathway. By contributing to the expanding knowledge in this field, paraganglioma research will almost certainly continue to be a powerful example of the way in which the study of a rare condition illuminates basic principles in biological and pathogenic processes, and facilitates the discovery of causes and remedies of more common forms of disease.