7. Summary

The carcinoma of the prostate is the most frequent tumor among men. Its appearance mainly with higher age has moved it to the focus of cancer research because of the rising life expectancy of the population.

For the analysis of this type of tumor the expression profiles of normal and tumor tissues from 54 patients were analyzed by Affymetrix chip hybridisations. After surgical removal the prostate was evaluated according to pathological standards and normal/tumor tissue was microdissected. After preparation the poly-A^+-RNA was linear amplified in repetitive rounds of cDNA synthesis and *in vitro* transcription and labeled with biotin for chip hybridisation. The hybridisation took place on an Affymetrix Oligochip carrying the sequences of 4000 genes. These genes had been previously identified as tumor-associated by metaGen Pharmaceuticals GmbH using bioinformatic analysis (Schmitt et al., 1999).

The chip data of 108 patient tissues and prostate cell lines were analysed by a special devolved algorithm and led to the identification of 124 sequences that were down regulated in prostate tumors. To this group of down regulated genes further 104 sequences with a significant up regulation in prostate tumors were selected and analyzed by cluster analysis.

The cluster analysis showed a distinct separation of tumor from its corresponding normal tissue with a sensitivity of 94% and a specificity of 72%. The algorithm used had a superior selectivity in comparison to the multiple published algorithm of Eisen et al. (1998). Separation of the tissues according to their histological classification was not possible in all cases, but this corresponds with observations within other groups analyzing different tissues (personal communication J. Staunton, Whitehead Institute, Cambridge USA). Clustering the tumors according to their grad and stage was not possible, because the histological features did not harmonize with the molecular profile.

This fact could lead to the identification of groups of tumors with similar molecular profiles and clinical developments and for the prediction of a possible development of an unknown prostate tumor. Individual treatments/therapies could then be applied to specific expression profiles. For such an extensive analysis beside an enhanced basis of experiments a complete patient history with follow-up data is necessary and that information is not available, to date.

For a detailed analysis of down regulated genes in prostate tumors, three were selected from this group and a fourth gene was chosen because of its similar function.

The down regulation of **WIF-1** in prostate tumors was very clear and could also be shown for breast and lung tumors. The reason for the down regulation is not known. WIF-1 is located in the region 12q14.3, this area has up to now not been correlated with any chromosomal aberration in prostate tumors. Immunohistochemical staining of prostate and bladder tumors confirmed the loss of expression in a subset of tumors. Low grade sections in the prostate showed a loss of protein expression with a simultaneous expression in high grade sections of the tumor. Further functional studies are needed to show how far the observed differential expression of WIF-1 has a suppressive role in different phases of the tumor formation.

From the Wnt-signal transduction pathway the gene **sFRP1** could be identified on the chip as a further gene with a similar function to WIF-1. sFRP1 shows no differential expression in prostate tumors, but is located in the region of 8p11.22 which is often lost in bladder tumors. The reduced gene expression could be shown in Northern blots and LOH analysis confirmed the loss of the region. Immunohistochemical analysis with an sFRP1-Antibody showed the loss of expression in 26% of the analyzed bladder tumors. sFRP1 is predicated to have a role in tumor development but overexpression and knock-out studies will have to confirm these presumptions.

As a third gene with a down regulation in prostate tumors **Chimerin-1** was chosen to be studied more closely because of its suppressing function on the signal transduction of the Ras-related Rho-Proteins. The differential RNA-expression for this gene could be confrimed.

For the adaptor protein **Ponsin** the differential RNA expression could be confirmed, but the protein expression was not differential between normal and tumor epithelia and this highlighted a possible methodical problem of manual microdissection. The LOH-analysis gave no significant result for a loss of the gene, while a clear loss could be identified for the more telomeric marker D10S1223.

The analysis of expression profiles of tumor tissues shows the possibility of the identification of novel tumor associated genes that also represent, due to their function, potential tumor suppressor genes. The analysis of the protein expression of Ponsin illustrates the importance of microdissection techniques that are able to isolate single cells.