Genetic characterization of dogs via chromosomal analysis and array-based comparative genomic hybridization (aCGH)*

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Summary
The results of cytogenetic and molecular cytogenetic investigations revealed similarities in genetic background and biological behaviour between tumours and genetic diseases of humans and dogs. These findings classify the dog a good and accepted model for human cancers and other genetic diseases. With the appearance of new studies and advances in canine genome sequencing, the number of known homologies in diseases between these species raised and still is expected to increase. In this context, array-based comparative genomic hybridization (aCGH) provides a novel tool to rapidly characterize numerical aberrations in canine tumours or to detect copy number aberrations between different breeds. As it is possible to spot probes covering the whole genome on each chip to discover copy number aberrations of all chromosomes simultaneously, this method is time-saving and cost-effective – considering the relation of costs and the amount of data obtained. Complemented with traditional methods like karyotyping and fluorescence in situ hybridization (FISH) analyses, the aCGH is able to provide new insights into the underlying causes of canine carcinogenesis.

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Molecular cytogenetics
There are two major ways in tumour biology to characterize the mechanisms involved in carcinogenesis. The classical approach is represented by conventional cytogenetics allowing the identification of structural and numerical aberrations of chromosomes of cultured tumour cells with a resolution of approximately 10 Mb providing a rapid overview of possibly altered chromosomal regions or whole chromosomes. Hereby, prepared chromosomes are banded and arranged according to standard nomenclature for the respective specimen facilitating the identification of structural and numerical aberrations. The International System for Human Cy-

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Molecular genetic analyses enable the characterization of gene structures and their tissue specific expression patterns pointing to possible quantitative and qualitative alterations in cancer development and progression, assumed that profound knowledge about structure and function of the gene of interest is available. Useful tools are cDNA- and BAC-libraries playing key roles in the molecular characterization of cancer and further diseases.

The combination of these two approaches resulted in the new field of molecular cytogenetics, with techniques such as fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), comparative genomic hybridization (CGH), array-based CGH and others. These fields thus open new possibilities for cancer development and progression with new chances for therapeutic strategies.

Array CGH

A novel method that gained significant influence in human routine diagnostic laboratory procedures is the array-based comparative genomic hybridization (aCGH). Its general principle bases on the hybridization of genomic DNA sequences to their unique complements. For the direct comparison of the DNA of interest with a reference DNA from healthy donors, the same amounts of these two DNAs are labelled with two different fluorophores. Commonly used fluorophores for DNA labelling are Cy3 and Cy5 or their AlexaFluor equivalents with the same wavelength. These differently labelled DNAs are then pooled and hybridized on glass slides spotted with DNA probes (Fig. 1). The intensity of fluorescence is correlated to the amount of complementary patients’ DNA for each spot (i.e. spots appear green, if reference DNA
The method of array-based comparative genomic hybridization (aCGH) is an elegant and – considering the relation of expenses and the amount of data obtained – cost-effective method to determine disease causing genomic imbalances in patients’ DNA that is widely used in human routine-diagnostic laboratories. The canine genome sequencing and its further characterization via fluorescence in situ hybridization (FISH) allowed the introduction of aCGH for the analyses of the canine genome. Thus it is possible to rapidly characterize canine diseases, such as cancer, and their underlying genetic causes. In addition, the aCGH can be used to detect copy number polymorphisms in different breeds.

Up to now there are two different major types of arrays available: at the beginning, the original arrays were spotted with probes derived from 87 BAC-clones (35). After the generation of the 7×7 dog genome assembly (12), canine CGH-arrays with BAC-clones covering wider parts of the genome were established with a median interval probe spacing of 10 (38) and 2 Mb (37). This spacing was narrowed by the development of an extended version in 2008, with a median probe spacing of 1 Mb (39). This refinement in resolution enables the detection of smaller genomic imbalances, and subsequent FISH-analysis with the appropriate BAC-clone reveals the true copy number. A tiling-path array with overlapping BAC-clones covering the whole genome is yet established only for humans (10), but a higher resolution for the canine genome can already be achieved by the second type of arrays, where the BAC-clones are replaced by synthetic DNA-oligomers with a length of 50–75 bp. These arrays are commercially available and display a median probe spacing of ~4.7 kb or ~2.7 kb depending on the manufacturer. The smaller size of the different probes compared to BAC-clones allows a more narrow spacing and thus the detection of smaller genomic imbalances, whereas the highest resolution has been achieved at a spacing of 1 kb and revealed 400 new copy number polymorphisms in different canine breeds (18).

Up to date – apart from the ones already mentioned – only few studies were published using aCGH with either BAC-clones or oligomers as probes for the characterization of the genomic structures of osteosarcomas, colorectal and transmissible cancer and non-Hodgkin lymphomas (1, 24, 34, 41), or to examine breed-specific copy number variations (17), but their number is expected to increase with the availability of the DNA-chips. Both types of arrays (oligomers as well as BAC-clones) are limited to the detection of numerical aberrations only, but especially chips with oligomer-probes can be used to define breakpoints of translocations, as they often go along with a gain or loss of genetic material. Taken together, the combination of aCGH with traditional methods such as karyotyping and FISH provides a powerful tool to gain new insights into chromosomal alterations that go along with tumour development. According to this, affected regions can be identified and their impact on tumourigenesis be further investigated, with the chance for the development of new therapeutic strategies.

Conflict of interest
The authors confirm that they do not have any conflict of interest.


