Project description:

The research area

Metastatic disease is the most significant factor for survival in cancer and the presence of disseminated disease will in most circumstances equal non-curable disease. Factors associated with the risk of developing distant metastases are mainly related to the primary tumor, such as stage and nodal involvement. However, these factors are simple and fail to include volumetric or molecular characteristics of the tumor that may influence the metastatic potential. Thus, to provide the best possible treatment and prognostication, the mechanisms responsible for development of metastasis and the factors associated with these are important to identify. Head-neck cancer squamous cell carcinomas (HNSCC) is an excellent model for studying these factors, as the disease is confined to the head and neck area (loco-regional) in most cases and an effective local treatment is offered to the majority of patients. Building upon previous work by our group, we propose a study to identify molecular and treatment associated factors involved in the metastatic process.

The project is divided into three specific aims

Aim 1: To determine prevalence and risk factors for distant metastatic disease in pharyngeal and laryngeal squamous cell carcinoma of the head-neck cancer

Aim 2: To establish the value of a newly developed gene expression profile we will characterize the primary tumor and the metastatic lesions in a cohort of pharyngeal and laryngeal cancer patients, in order to define stem cell markers, propose a high-risk gene profile and distinguish a distant metastatic recurrence from HNSCC from a new primary SCC

Aim 3: To detect circulating tumor cells (CTCs) in head-neck cancer patients (human papilloma virus (HPV)-positive SCC of oropharynx), and establish the significance of radiation therapy on the recruitment of CTCs

The project

Subproject 1:

The epidemiology of distant metastases in HNSCC and the influence of tumor volume on the risk of developing distant metastases

Previous studies of distant metastases in head-neck cancer have primarily been hospital-based with the inborn risk of selection bias. We propose a population-based retrospective cohort study to determine the risk and temporal development of distant metastases following treatment for HNSCC. We will utilize a previously characterized dataset from Denmark, supplemented by data from the remaining Danish centers.

The Danish Head-neck Cancer Group (DAHANCA) clinical database contains information on etiological factors, UICC stage, P16 (a proxy of HPV status), treatment, follow-up, recurrence and cause of death. It includes all cases of HNSCC since 1991 (>33,000) and the coverage is close to 100%. This study cohort concerns the HNSCC subsites of the throat (pharynx and larynx) while the cancer of the oral cavity has been excluded due to suboptimal registration in earlier years. It includes all patients from 2007-2017, excluding patients from Greenland and Faroe Islands. The approx. number of patients in this cohort is 7000.
The completeness of metastatic disease registration will be validated using linkage to the Danish Pathology Register, and in case of doubt patient charts will be reviewed. Within the cohort we will identify patients with metastatic disease at presentation (so-called M1 patients) of which there are approx. 4 % of the cohort and patients with distant recurrence after treatment, approx. 8-10 %. Thus, the total number of patients with metastatic disease will be around 800-1000 patients in the whole cohort. Tumor volume in patients without metastatic disease at presentation (M0) will be obtained from treatment planning systems from the six Danish centers treating head-neck cancer. The available variables in the database will allow an analysis of the temporal trend and risk factors for both metastatic disease at presentation and metastatic recurrence after primary treatment. The analyses will be performed using a Cox regression model with adjustment for relevant confounders.
The analyses will generate:
a) Epidemiologic trends of distant metastases at presentation or as a recurrence after HNSCC in a national cohort
b) Estimate the value of tumor volume and UICC stage in estimating the risk of distant metastases as a recurrence after HNSCC

<u>Subproject 2:</u>

Gene sequencing of primary HNSCC and suspected metastases

The cohort of patients is identical to aim 1 but concerns only patients with distant recurrences after primary treatment (approx. 400-500 patients). Using Patobank, patients with useful tissue samples from both the primary tumor and the metastatic lesion(s) will be identified. Based on a local survey at Rigshospitalet approximately 25-50% of patients will have useful tissue samples, equaling 100-250 patients in the cohort of which the majority will have lung metastases. Tissue blocks from both the metastatic lesion and primary tumor will be obtained and due to logistic limitations we expect to collect approx. 100 tissue block pairs.

The 100 pairs of primary tumors and distant recurrences will be analyzed genetically for TP53 and HPV subtype. First, this will allow an analysis of the concordance between the genetic profiles in primary tumors and in the assumed metastatic lesions. Secondly, in patients with concordance

between primary tumor and metastatic profiles, a high-risk genetic profile will be developed, based on variations in the gene profile and stem cell markers. This profile is hypothesized to predict which patient will develop metastatic recurrence. To test this hypothesis, all primary tumors will be compared to primary tumors of a historical cohort, and the ability of the genetic profile to predict the risk of metastatic recurrence will be tested. Additionally, we will be able to report the concordance with the historical clinical classification and registration of recurrences.

Subproject 3:

Circulating tumor cells (CTCs) in head-neck cancer

Fifteen patients with HPV-positive head-neck cancer planned for radiotherapy with curative intent and a tumor volume of at least 5 cm3, will be asked to deliver whole blood (20 ml) prior to radiotherapy and 2, 4 and 24 hours after the first treatment fractions and halfway through treatment (16^{th} fraction). Fifteen healthy age and gender-matched persons will serve as controls. The samples will be transported to Centre for Medical Parasitology, University of Copenhagen, where analysis will be performed as described earlier. CTCs will be validated using HPV detection. CTCs per 5 ml of blood will be compared between HNSCC patients and healthy controls at the different time points.