Chapter 2

RNA/DNA BASED DETECTION OF MINIMAL RESIDUAL HEAD AND NECK CANCER

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Abstract

The prognosis of head and neck cancer is largely determined by the radicality of treatment: residual tumour cells will grow out and develop in manifest local recurrences, regional recurrences and distant metastases. Classical diagnostic methods such as radiology and histopathology have limited sensitivities, and only by molecular techniques minimal residual cancer or disseminated tumour cells can be detected. In tissue samples containing the normal tissue counterpart of a tumour only (pre)cancer cell-specific markers can be exploited, whereas in other samples tissue-specific markers can be used. Currently, there are two main methodologies in use, one based on antigen-antibody interaction, and the other based on amplified nucleic acids. The most commonly used nucleic acid markers are mutations or alterations in tumour DNA (tumour-specific markers) or differentially expressed mRNA (tissue-specific markers). The limits of detection of these molecular assays can reach levels of a single tumour cell in a background of 2×10^7 normal cells. The assays are, however, often complex, demand a large experience and are usually laborious. Nevertheless, the data collected with these assays enable the elucidation of unexplained clinical phenomena. Further technical developments might allow implementation in clinical practice once the relevance has been assessed in large prognostic trials with long-term follow-up. In this chapter a number of the molecular assays used for (pre)cancer cell detection in head and neck cancer patients will be presented.

1. SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Cancer of the head and neck is the fifth most common incident cancer throughout the world. Head and neck cancer accounts for 42,000 new cases of cancer and 12,000 deaths per year in the USA. The five-year survival rates are generally about 50% to 70% (1–3). About 95% of all head and neck cancers in the western world are squamous cell carcinomas (HNSCC) (3, 4). The treatment failure of head and neck cancer is determined by local recurrences, regional recurrences in the lymph nodes above the clavicle, distant metastases and second primary tumours (5, 6). In former times the fate of the patient with head and neck cancer

K. Pantel (ed.), Micrometastasis, 19–45.

was determined by the local tumour process which rapidly destroyed the vital anatomical structures in this region (7). After the introduction of surgical techniques for resection of the tumour at the primary site, recurrence in the lymph nodes above the clavicles gained importance (8). In 1906 the neck dissection was introduced by Crile (9) as a treatment methodology for the regional lymph nodes. Improvement of surgical techniques, as well as the introduction and development of radiotherapy and chemoradiotherapy, further increased the locoregional control twofold. However, the improved locoregional control resulted in only a moderately increased survival rate of HNSCC patients, as also the rate of distant metastases increased twofold (10–14). Recently, long-term results of the treatment of HNSCC at the base of the tongue with surgery and radiotherapy were published with a median follow-up of 36 months, showing successful local control in 89% of the cases and neck control in 96% of the cases. The most common site of treatment failure was the development of distant metastases in 24% of the cases (15). These long-term results demonstrate the possibilities of modern diagnostic and therapeutic modalities. It should be mentioned, however, that the risk for locoregional recurrences strongly depends on the site and stage of the tumour, as well as the therapeutical management. For example, tumours in the larynx have a much better prognosis than tumours in the floor of the mouth mainly due to the well-defined anatomical borders of the larynx.

Lymph node metastasis is the most important mechanism in the spread of HNSCC. In general, lymph nodes are rather poor barriers to tumour cells (16), and clinical practice has shown that lymph nodes quite often appear to be a fertile soil for tumour growth. The presence of lymph node metastases is an important prognosticator in head and neck cancer. The presence of lymph node metastasis reduces the expected survival by approximately 50% (17–20). The frequency of lymph node metastasis is dependent on the site and the T stage of the tumour (see below). For example, T1, T2 and T3 oral tongue carcinomas have a risk for nodal involvement of 18%, 33% and 60% respectively, whereas T1, T2 and T3 floor of the mouth carcinomas have a risk of 38%, 65% and 71% respectively (21, 22). Not only the presence, but also the number of nodal metastases, the level in the neck, the size of the nodes and the presence of extranodal spread are important prognostic factors (19).

The neck nodes are divided into five anatomically defined levels, all of which are dissected in a comprehensive neck dissection. The lymph nodes in the different levels have different rates for metastatic involvement (21). The levels 1, 2 and 3 are more often affected by lymph node metastases than levels 4, 5 and 6 (22). Most tumours have a well-defined and predictable pattern of metastasis to the neck (23–25). For example, tumours in the anterior floor of the mouth disseminate most frequently to level 1. Many studies on this subject have been published, some deriving the data from unreliable clinical findings (26), and others from pathological reports obtained from therapeutical (27, 28), or elective neck dissections (23, 29). From the many studies published it can be derived that primary carcinomas from all sites can eventually spread to all levels of the neck (30).