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## **New developments in analysis of ocular surface diseases|Nieuwe ontwikkelingen in analyse van ziekten van het oogoppervlak**

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# CHAPTER 7

## **A NEW CELL LINE FROM A RECURRENT CONJUNCTIVAL MELANOMA.**

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The incidence of conjunctival melanoma is about 0.2-0.8/1.000.000 cases each year.<sup>1;2</sup> As a consequence our knowledge about this tumor is limited but cell lines derived from these tumours will give more information about them. So far only three conjunctival melanoma cell lines have been described.<sup>3;4</sup> The first cell line, IPC 292, was described in 1993 by Aubert et al.<sup>4</sup> Recently Nareyeck et al. developed two additional conjunctival melanoma cell lines (CRMM1 and CRMM2).<sup>3</sup> We wish to report a further, fourth, conjunctival melanoma cell line CM2005.1.

The conjunctival melanoma cell line CM2005.1 was established from tumor material derived from a 84 year old male. The primary tumor was located at the medial side of the inner upper eyelid adjacent to the cornea. The tumour was treated initially by local excision together with adjuvant Iridium 192 brachytherapy. After three years the tumour reappeared on the inner side of the lower eyelid. The tumour extended into the nasal cavity and disseminated further. The patient died one year later from the melanoma which had originated in the conjunctiva. Both the primary tumor and the local recurrence were histologically proven conjunctival melanomas.

From the local recurrence two small tumor specimens were available for cell culture. The tumor material was cut into small pieces with scalpels and transferred to several culture plates. Culture plates contained 10 mL/dish RPMI 1640 (Invitrogen-Gibco, Groningen, The Netherlands) supplemented with 10% FCS (Hyclone, Logan, UT), 100 IU/mL penicillin (Invitrogen-Gibco), and 100 µg/mL streptomycin (Invitrogen-Gibco). Cultures were incubated at 37°C in a humidified atmosphere and a CO<sub>2</sub> content of 5% in air and the culture medium was refreshed every 72-96 hours. After four months a stable cell line had been developed, which we were grown for over 22 passages. Cell doubling time was measured three times by culturing 100.000 cell/plate. The cell count was measured again at days 1, 2, 3, 4, and 5, which resulted in a cell doubling time around 35 hours (Figure 1).

To establish the melanocytic origin of the cultured cells, cytopins were undertaken and immuno-histochemically stained for S100, MelanA, NKI-C3, and HMB 45.

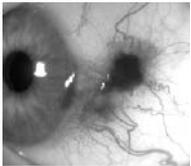
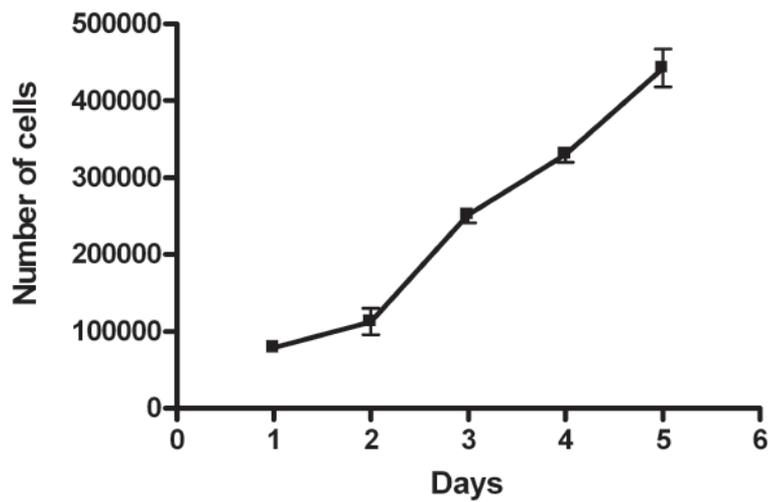
Immunohistochemical reactions were performed using the streptavidin-biotin method. Of the cultured cells 95-100 % were positive for all four primary antibodies thereby proving their melanocytic origin. MelanA, HMB 45, NKI-C3 stained the cultured cells intensively, the S100 labeling was slightly less intense.

To assess the karyotype of cell line CM2005.1, cytogenetic analysis was performed. Chromosome preparations were made according to standard procedures and stained to obtain R or Q banding. Cytogenetic abnormalities were described in accordance with the ISCN.<sup>5</sup>

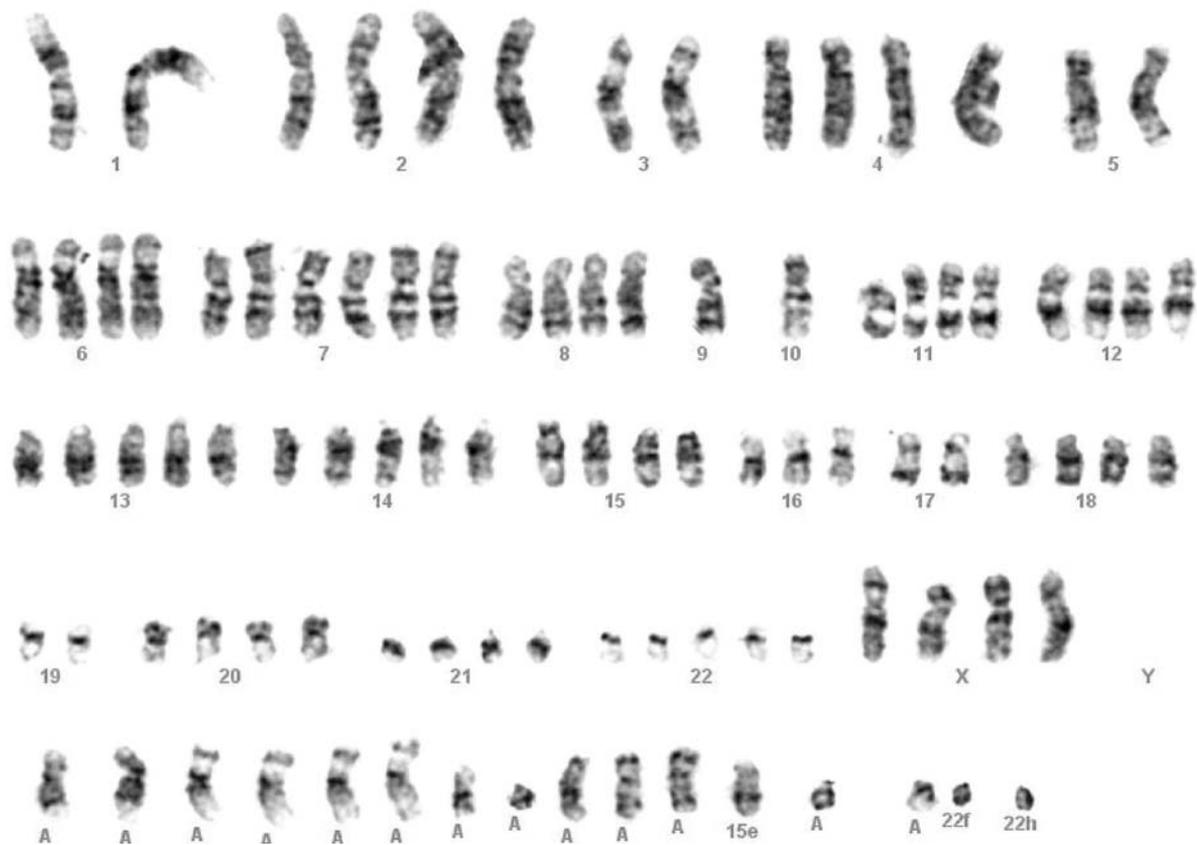
The following karyogram was found in the majority of cells: 83~96, XX, +X, -Y, -Y, -1, -1, -3, -3, -4, -5, -5, -6, +7, +7, +del (7) (q22), -8, -9, -9, -10, -10, del (11) (q23)x2, -12, +13, +13, +14, -16, -17, -17, -18, -19, +20, -21, -21, -21, -22, +12~16mar (see Figure 2).

Cell line CM2005.1 was derived from a recurrent conjunctival melanoma that showed recurrence after excision and brachytherapy, which could explain the distorted karyogram and the relative high cell doubling time of this cell line. However, the cytogenetic analysis was performed four months after start of the culture, which could have influenced the karyogram and may therefore not represent the original tumor. Nareyeck et al. found cell doubling times around 60h for their conjunctival melanoma cell lines CRMM-1 and CRMM-2.<sup>3</sup>

This fourth conjunctival melanoma cell line is now available for research, and can hopefully attribute to the expansion of our knowledge of conjunctival melanomas.



**Figure 1.** Growth curve of conjunctival melanoma cell line CM2005.1. Showing the number of cells at various time point during culture. Cell doubling time was around 35 hours.



**Figure 2.** Karyogram of cell line CM2005.1. A very complex karyogram with gains, deletions and rearrangements of almost all chromosomes was observed in the majority of cultured cells.

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