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ONCOMODULATORY PROPERTIES OF THE HUMAN CYTOMEGALOVIRUS-ENCODED RECEPTORS US28 AND UL33

The work described in this thesis was performed at the Leiden/Amsterdam Center for Drug Research (LACDR), Faculty of Sciences, Division of Medicinal Chemistry, VU University Amsterdam, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

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Oncomodulatory properties of the human cytomegalovirus-encoded receptors US28 and UL33

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door

Ellen Vera Langemeijer

geboren te Almelo

Promotor: prof.dr. M.J. Smit
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contents

aim and outline of this thesis

A large percentage of the human population is infected with herpesviruses. Although these viruses are usually harmless at primary infection, they all possess the ability to remain in a latent stage in the infected host. Upon malfunctioning or suppression of the host immune system, these viruses can be reactivated which can lead to serious pathological conditions [1, 2]. The HHV5, or human cytomegalovirus (HCMV), is known to cause serious diseases like pneumonitis, retinitis and hepatitis in immune-compromised individuals, like transplant recipients and AIDS patients. More recently it also appeared to be involved in the development of vascular and proliferative diseases [3, 4].

HCMV, like other herpesviruses, contains integrated host genes resembling chemokine receptors in their genome, modified to their own benefit. Unlike human chemokine receptors, these hijacked viral variants in general contain the capacity to signal in a constitutive manner. For HCMV four chemokine receptors (US27, US28, UL33 and UL78) have been described. The potential role of US28 and UL33 in rewiring cellular signaling is described in this thesis.

Constitutive signaling displayed by viral chemokine receptor homologs results in modulation of intracellular signaling pathways. This will have profound effects on the (patho-) physiological behavior of the infected cell, as was demonstrated for ORF74, the KSHV-encoded chemokine receptor. By *in vitro* as well as *in vivo* studies this receptor was shown to induce cellular transformation, leading to tumor formation in nude mice [5]. A comparable observation using US28-expressing NIH-3T3 cells was made; US28 induces *in vitro* proliferation as well as tumor formation *in vivo* [6].

The **aim of this thesis** is to increase our understanding on how HCMV-encoded receptors US28 and UL33 modulate cellular signal transduction.

We show that the constitutively active chemokine receptor US28 displays oncomodulatory properties, providing molecular links between viral infection and subsequent pathologies. US28-mediated signal transduction induces alternative gene expression profiles via activation of COX-2 (**Chapter 2**) and β-catenin (**Chapter 3**). These important cancer signaling pathways activated by US28 are investigated in detail in *in vitro* studies including HCMV infection studies. The cellular pathophysiological effect of this vGPCR-triggered signaling is described in particular in relation to proliferative responses. In **Chapter 4** signal transduction pathways induced by the HCMV-encoded receptor UL33 are investigated. We show that UL33 also act as an oncomodulator by rewiring cellular signaling in NIH-3T3 expressing cells, and thus potentially in proliferative diseases. Epigenetic aberrations induced by the virus-encoded chemokine receptor US28 are investigated for the first time. In **Chapter 5** we show that hypomethylation of the cell cycle gene cyclin D1 is a proliferation-related epigenetic regulation induced by US28.

Summarized, this thesis focuses on the oncomodulatory properties of two viral receptors US28 and UL33. In order to understand how these two β-herpesvirus receptors influence cellular signal transduction, transiently transfected cells, stably-expressing US28 or UL33 cells as well as virus-infected cells were subjected to detailed signaling studies.