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TOLL-LIKE RECEPTOR EXPRESSION IN CELL LINES AND PRIMARY TUMOUR MATERIAL

Anti-inflammatory pathways such as those recruited in response to the activation of the glucocorticoid receptor by ligands like Dexamethasone, might have an adverse effect on the response of tumour cells to therapy. In a prophylactic mode, mechanisms that reduce inflammation might be of significant benefit in reducing the likelihood of tumour development. Toll-like receptors form a part of the pattern recognition receptors that are responsible for innate immunity in the cells of the immune system. Recent finding of Toll¬like receptors in many somatic tissues and especially in the various forms of tumors provide novel ap¬proaches as for the development of specifically targeted chemotherapeutic agents. Current research project focused on the investigation of the expression patterns of the Toll-like receptors in the primary meningioma biopsies and cell lines of different origin.

Although anti-inflammatory responses in the immune system involve direct downregulation of the leukocyte activity and reduction in the cytokine production [1]. Toll receptor (TLR) proteins that are involved in a fist-line response to infectious agents, such as bacteria and fungi (as described in an earlier section of this thesis), are also regulated. Therefore, in gene profiling studies, glucocorticoids have been shown to alter the expression of Toll 2 and Toll 4 [2]. A rather fundamental question that might be asked, therefore, is whether tumour cells express functional toll receptors and whether this property can be exploited for therapeutic use. To address this, PCR studies were conducted on the expression of toll-like receptors in tumour cell lines and primary human tumour material.

Expression of Toll receptors in human cell lines

A PCR protocol was established based on recent studies on the expression of Toll receptors in normal human cells [3]. Primer sequences were selected and a standard PCR reaction was performed on cDNA generated from RNA extracted and from tumour cells In order to check the specificity of the primers, a PCR reaction was first performed on the chronic myeloid leukemia cell line, K562. Fig. 1 shows that this cell line is indeed of leukocyte in origin due to the expression of the pan-leukocyte marker, CD45 and with the exception of TLR 8 and 9, all other receptors are clearly expressed. The sizes of the PCR products were determined by comparative analysis with known molecular size markers and were con¬firmed to be consistent with product sizes reported in the literature by using a UV-Itec gel-documenta¬tion and band analysis system.

All PCR products were checked with a gel-docu¬mentation system and sizes of the PCR products corresponding to the predicted product sizes are as indicated below. Slight variation in the product siz¬ing is due to the imperfection and artifacts during electrophoresis. Fig.1. is shown as a negative. Prod¬ucts were compared to the known size markers in order to determine their size (Table 1).

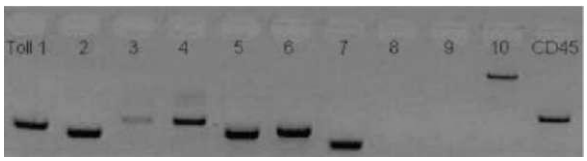

Fig. 1. TLR expression in the CD45+K562 human chronic myeloid leukemia cells

Table 1. **Predicted and actual sizes of the RT-PCR products for the TLR receptor expression analysis**

	Toll 1	Toll 2	Toll 3	Toll 4	Toll 5	Toll 6	Toll 7	Toll 8	Toll 9	Toll 10
Predicted size	104	93	114	110	89	96	80	123	452	207
Actual size	105	96	114	111	87	94	83	125	460	214

Actual size of the PCR product was identified by comparison to a known size ladder marker (Hyper-ladder 100bp) using a gel-doc. system.

Colorectal cancers are commonly associated with chronic inflammatory conditions and informa¬tion on receptor expression in cell lines derived from this neoplastic condition should reflect the pat-tern of Toll-like receptor expression in the meningi-oma tumour if their onset is associated with inflam¬mation. However this is a speculative idea that is based on the assumptions that innate immune re¬sponses to the chronic inflammation mediated via toll-like receptors are essentially identical in the tis¬sues of different origin. COLO and CACO cell lines

were initially screened with the PCR assay for the Toll-like receptor expression. Table 2 shows that as many as 6 members of the Toll receptor family (TLR 1-6) were expressed in COLO cell line and 8 receptors (1-6, 9 and 10) were found in the CACO cells.

Further studies on other cells and tumour cell lines indicated that Toll receptors were expressed in a wide range of cells of unrelated origin, although with some subtle differences in the expression patterns.

The data presented in Table 2 represents a consensus from two different passages of tumour cells where each PCR reaction, for each receptor, was performed in triplicate. The pan-leukocytic marker, CD45, was included in the screening for the optimisation of the thermal cycling conditions as it was intended for later screening of primary human tumour-derived cells in order to exclude the possibility of leukocyte infiltration in the tissue. This step was considered important due to the fact that cells of the immune system are known to have high-level expression of all members of the Toll-like receptors and this might invalidate studies on RNA extracted from sections of primary tumour material, where a mixed population of cells is present.

Toll-like receptor expression in primary human tumour material

The data presented above on the presence of Toll-like receptor members in a range of tumour cell lines further substantiates the link between chronic inflammatory conditions and the establishment and the promotion of cancer. More importantly, it establishes a link between initiators of inflammatory reactions and a direct response at the level of the tumour cell. There are always potential problems using tumour cell lines, as due to genetic and epigenetic drifts and senescence changes over the time that cells are kept in culture, receptor expression patterns may not always reflect those of primary tumour material. As access was available to primary tumour material through the Department of Neuro-Endocrinology at the Max Planck Institute of Psychiatry in Munich, studies were conducted on primary meningioma. Due to the complexity of Meningioma tumour pathophysiology (see Appendix for a screening of Meningioma tissue Histopathology), a great deal of care was devoted during sample preparation for the RT-PCR screening, in order to avoid any possibility of leukocyte contamination. Dissections of the tumour biopsies were performed under a binocular microscope and a fresh set of disposable scalpels was used for each sample. Using these procedures, meningioma biopsies were selected to be screened for the expression of Toll-like receptor family. RT-PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualized under UV light. Fig. 2. shows the TLR expression pattern in two different meningioma tumours (female and male-derived tumours respectively) as determined by RT-PCR.

By screening 8 more tumours in this way, it soon became apparent that a large proportion of the TLR receptors were expressed in the meningioma tumours with Toll-1, 2, 3, 4, 5, 6, 9, and 10 being present in most of samples (see Table 3). Some tumours showed subtle differences in the expression patterns and no samples had a CD45 positive band. The latter finding indicated that the Toll receptor expression detected in the tumour samples was not due to overwhelming infiltration by cells of the immune system as was genuinely due to the expression of the receptor by the cells that constituted the tumour.

Table 2. **Expression of Toll-like receptors in the human cancer cell lines of different origin as determined by RT-PCR**

		Toll like receptors										CD45
Cell line	**Origin**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
COLO	Colorectal	+	+	+	+	+	+	-	-	-	-	-
CACO	Colorectal	+	+	+	+	+	+	-	-	+	+	-
U87MG	Glial	+	+	+	+	+	+	-	-	+	+	-
LOVO	Colorectal	+	+	+	+	+	+	-	-	-	+	-
MCF-7	Breast	+	+	+	+	+	+	-	-	+	+	-
Eahy 962	Endothelial	+	+	+	+	+	+	-	-	+	+	-
K562	Hematopoietic	+	+	+	+	+	+	+	-	-	+	+

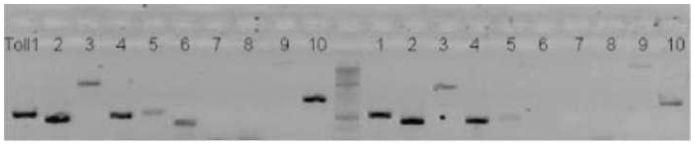

Fig. 2. Comparison of the expression of Toll-like receptors 1-10 in primary human meningioma tumour material derived from one female and one male patient. PCR product sizes were consistent with those obtained for the study on different cell lines

Table 3. **Grouped analysis of the Toll receptor expression in meningioma tumour tissue**

		Toll receptor expression										CD45
Tumour number	Male/Female	1	2	3	4	5	6	7	8	9	10	
1	F	+	+	+	+	+	+	-	-	-	-	-
2	M	+	+	+	+	+	+	-	-	+	+	-
3	M	+	+	+	+	+	+	-	-	+	+	-
4	F	+	+	+	+	+	-	-	-	+	+	-
5	F	+	+	+	+	+	+	-	-	+	+	-
6	M	+	+	+	+	+	+	-	-	-	+	-
7	F	+	+	+	+	-	+	-	-	+	+	-
8	F	+	+	+	+	-	+	-	-	+	+	-
9	F	+	+	+	+	+	+	-	-	+	+	-
10	M	+	+	+	+	+	-	-	-	+	+	-

The results presented in Table 3 are consensus values from duplicate PCR reactions for each receptor.

Data presented in Table 3 on the expression of Toll-like receptors in primary human meningioma tumour cell material and the data derived from the cell lines supports the view that Toll receptor expression is a common finding in human tumour cells. Whether receptors are constitutively expressed or their expression is induced or decreased in response to anti-inflammatory agents is another question that should be answered.

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ЕКСПРЕСІЯ TOLL-LIKE-РЕЦЕПТОРІВ У КЛІТИННИХ ЛІНІЯХ ТА ДЕЯКИХ ЗЛОЯКІСНИХ ПУХЛИНАХ

Протизапальні механізми, що регулюються через глюкокортикоїдний рецептор та активуються такими лігандами, як дексаметазон, можуть відігравати негативну роль у відповіді пухлини на хіміотерапію. Водночас профілактичні заходи, що зменшують ризик виникнення хронічних запальних процесів, можуть зменшити ризик неоплазматичного перетворення та розвитку ракової пухлини. Toll-like-рецептори, які активуються широким спектром структурно споріднених молекул, є основними медіаторами вродженого імунітету. Нещодавні дослідження показали наявність Toll-like-рецепторів у соматичних тканинах, а також у декількох типах пухлин, що надає нові можливості у розробці новітніх протипухлинних препаратів. Проект сфокусовано на вивченні експресії Toll-like-рецепторів у біоптатах менінгітом та у клітинних лініях різного походження.